

Glucocorticoids and bone: consequences of endogenous and exogenous excess and replacement therapy

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1 Title: **Glucocorticoids and bone: consequences of endogenous and**
2 **exogenous excess**

3

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14

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16

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18

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34

35 Abstract:

36 Osteoporosis associated with long-term glucocorticoid therapy remains a common and serious bone
37 disease. In addition, in recent years it has become clear that more subtle states of *endogenous*
38 glucocorticoid excess may have a major impact on bone health. Adverse effects can be seen with
39 mild systemic glucocorticoid excess but there is also evidence of tissue-specific regulation of
40 glucocorticoid action within bone as a mechanism of disease. This review article will examine a) the
41 role of endogenous glucocorticoids in normal bone physiology, b) the skeletal effects of endogenous
42 glucocorticoid excess in the context of endocrine conditions such as Cushing's disease and
43 autonomous cortisol secretion (subclinical Cushing's syndrome), and c) the actions of therapeutic
44 (exogenous) glucocorticoids on bone. We will review the extent to which the effect of
45 glucocorticoids on bone is influenced by variations in tissue metabolising enzymes and
46 glucocorticoid receptor expression and sensitivity. We will consider how the effects of therapeutic
47 glucocorticoids on bone are complicated by the effects of the underlying inflammatory disease being
48 treated. We will also examine the impact that glucocorticoid replacement regimens have on bone in
49 the context of primary and secondary adrenal insufficiency.

50

51 Precis:

52 We reviewed literature relating to the effects of glucocorticoids on bone. This included the impact of
53 endogenously synthesised and therapeutically administered glucocorticoids on bone and bone cells.

54

55 I. Introduction

56 Glucocorticoid induced osteoporosis (GIOP) remains an important and common clinical problem.
57 GIOP was first recognised in patients with Cushing's disease or other states of endogenous
58 glucocorticoid excess.¹ However, since the introduction of therapeutic glucocorticoids over 60 years
59 ago, GIOP is now much more commonly seen in people treated with therapeutic glucocorticoids.² It
60 is well established that therapeutic glucocorticoid treatment is associated with significant loss of
61 bone density, deterioration of bone structure and substantial increases in fracture risk.^{3,4} The
62 condition appears to behave in many ways distinct to that of age-related or postmenopausal
63 osteoporosis and, as such, is regarded as a distinct metabolic bone disease.⁵

64 The study of GIOP is complicated by the almost universal involvement of an underlying, usually
65 inflammatory disease, as the reason for glucocorticoid treatment in the first place.^{5,6} These
66 underlying illnesses are rarely incorporated into animal models examining the pathogenesis of GIOP.
67 Various treatments have been evaluated for GIOP in the clinical setting but usually only after these
68 have proven effective in the context of postmenopausal osteoporosis. Trials in GIOP are generally
69 powered based on BMD changes rather than fracture risk reduction. All current treatments for GIOP
70 have significant limitations in terms of effectiveness and risk of adverse effects.

71 In addition to the clear evidence that high levels of therapeutic glucocorticoids do harm to bone
72 there is increasing evidence that more subtle states of endogenous of glucocorticoid excess
73 detrimentally impact on bone.⁷ The main focus of this research has been the impact of subclinical
74 endogenous hypercortisolism (also known as sub-clinical Cushing's syndrome or autonomous
75 cortisol secretion), a condition characterised by autonomous cortisol secretion usually by one or
76 more adrenal cortex nodules. There is current debate regarding how prevalent this condition is and
77 how significant its impact is on bone but many studies indicate that the effects on bone can be
78 substantial.⁷ It is less clear how to investigate and manage bone loss and extra-skeletal
79 manifestations in subclinical endogenous hypercortisolism.

Comment [M1]: ? add graphical abstract here?

80 There is now considerable evidence that glucocorticoid action can be modulated by various
81 mechanisms at a tissue level. These mechanisms include variations in the expression and sensitivity
82 of the glucocorticoid receptors⁸, export of steroids out of the cell by transmembrane transporters⁹
83 and enzymatic metabolism of glucocorticoids to more or less active forms.¹⁰ In particular, there has
84 been interest in the role of the 11 β -hydroxysteroid dehydrogenases (11 β -HSDs) which interconvert
85 the active glucocorticoids cortisol and corticosterone with their inactive counterparts cortisone and
86 dehydrocorticosterone.¹⁰ These enzymes appear to influence bone cell differentiation and function
87 and changes in enzyme expression have been implicated in the development of some aspects of
88 glucocorticoid induced bone loss. Excessive tissue glucocorticoid action in the presence of normal
89 circulating levels of glucocorticoids might thus play a more generalised role in other forms of
90 osteoporosis not traditionally associated with glucocorticoid excess.

91 The issue of whether glucocorticoid levels are sufficient, inadequate or excessive for bone health is
92 relevant to the treatment of states of adrenal insufficiency such as Addison's disease or
93 hypopituitarism. Evidence suggests that historically, glucocorticoid replacement regimens were
94 excessive in many people and this is likely to have detrimentally impacted on bone health. More
95 contemporary (and lower) replacement glucocorticoid doses appear to have less of an adverse
96 impact on bone in terms of bone density and biochemical markers. However, whether this translates
97 into reduced fracture risk is unclear.

98 This review will therefore examine the role endogenous glucocorticoids play in normal bone
99 physiology, examine the skeletal effects of endogenous glucocorticoid excess in the context of
100 endocrine conditions such as Cushing's disease and autonomous cortisol secretion, and explore the
101 actions of therapeutic glucocorticoids on bone. Based on a MedlineTM publication search within the
102 last five years (to February 2018) supplemented by earlier studies of continuing significance we
103 review how the effect of glucocorticoids on bone is influenced by tissue metabolising enzymes and
104 glucocorticoid receptor expression. We will consider how these effects are complicated by

Comment [RSH2]: Could use a figure here

105 inflammation. We will additionally examine the impact that glucocorticoid replacement has on bone
106 in the context of adrenal insufficiency.

107

108 II Mechanisms of action of glucocorticoids on bone

109 This section outlines how glucocorticoids have their effect on bone. Evidence primarily based on
110 mouse models suggests that the main adverse effects of high levels of glucocorticoids on bone are
111 through direct effects on cells involved in bone remodelling; osteoblasts, osteocytes and osteoclasts.
112 Mechanisms such as impaired cellular proliferation, increased apoptosis, altered autophagy and
113 changes in RANKL/OPG, wnts/sclerostin expression have all been proposed to be important
114 mediators of these effects. These mechanisms have been examined in animal models of disease and
115 to some extent in human samples. We will conclude this section by discussing evidence that some of
116 the adverse effects of glucocorticoids on systemic fuel metabolism are mediated through the
117 skeleton.

118 An important consideration when interpreting the literature relating to glucocorticoid effects on
119 bone is to appreciate the significance and relationships of the various forms of glucocorticoids that
120 have been studied or implicated in disease. The main glucocorticoid secreted from the adrenal
121 cortex in humans is cortisol. When administered therapeutically, cortisol is referred to as
122 hydrocortisone. A smaller amount of corticosterone (about 5-10% that of cortisol) is also secreted
123 from the human adrenal cortex.¹¹ Although traditionally considered to have a minor role in human
124 physiology recent work examining the selective export of cortisol and corticosteroids from the cell
125 suggests that corticosterone secretion could be important over and above the secretion of cortisol.⁹
126 In the mouse and rat corticosterone is the main glucocorticoid secreted from the adrenal due to the
127 absence of the 17 α -hydroxylase enzyme in the adult adrenal gland in rodents.¹² Cortisol and
128 corticosterone have direct and similar actions at the glucocorticoid and mineralocorticoid receptors
129 but in classical mineralocorticoid target tissues (kidney, colon, salivary and sweat glands) these
130 glucocorticoids are inactivated by the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2)
131 to cortisone and dehydrocorticosterone respectively. These steroids lack activity at the level of the
132 GR or MR but can be reactivated to cortisol and corticosterone by 11 β -hydroxysteroid

Comment [RSH3]: Again a figure here could be useful. Could we tie it into HPA axis, with relative circulating levels and the 1st pass metabolism of pred in liver

Agree we need a figure

dehydrogenase type 1 (11 β -HSD1) enzyme which is expressed in a range of tissues, in particular in the liver but also in bone. Prednisolone and prednisone are the most widely used oral glucocorticoids. As with cortisol and cortisone these compounds differ by just a hydroxylation at position 11 of the steroid ring with prednisolone being the active form and prednisone the inactive form. In practice orally administered prednisone and prednisolone have similar properties in vivo since prednisone is efficiently converted to prednisolone by hepatic 11 β -HSD1 activity on first pass metabolism in the liver.

Endogenous glucocorticoids have the potential to bind to either the classical glucocorticoid receptor (GR) or the mineralocorticoid receptor (MR). The MR is also referred to as the type 1 or high affinity GR since the affinity of the MR for cortisol and corticosterone is 10 times higher than that of the GR.¹³ As discussed above, the main factor influencing endogenous glucocorticoid binding to the MR is the presence of 11 β -HSD2. As a further complication, the synthetic glucocorticoid dexamethasone only binds to the GR and not the MR. These differences have important implications when interpreting differences between studies examining the mechanisms underlying GIOP.

The downstream cellular consequences of glucocorticoid receptor binding have been reviewed in detail elsewhere.^{14,15} These mechanisms will be discussed in each section where specifically relevant to glucocorticoid actions on bone.

II.I Effects on osteoblasts, osteocytes and osteoclasts

Glucocorticoids have direct effects on specific tissues but also exert their effects through indirect mechanisms, e.g. through the regulation of endocrine signalling pathways. The extent to which glucocorticoid induced bone loss is mediated through direct effects on the cells which coordinate bone metabolism (osteoblasts, osteocytes, osteoclasts and their respective precursors) has been debated. Bone cells are clearly very sensitive to glucocorticoids in vitro and in vivo. In vivo mouse

157 models that have attempted to examine this question show that the effects of glucocorticoids on
158 bone are primarily through direct actions on bone cells and bone remodelling. The situation
159 regarding the important clinical manifestations of GIOP in humans, fractures, might be different
160 since glucocorticoids can influence falls related factors such as muscle strength that are difficult to
161 replicate in mice.

162 Whether or not there is a single primary or dominant target of glucocorticoids accounting for the
163 effects on bone remains unclear. This is not helped by the wide variety of mouse models which vary
164 according to strain; age; sex; skeletal site examined; and type, dose, duration and route of
165 glucocorticoid administration.^{16,17} Furthermore, the effects of glucocorticoids are not consistent
166 across skeletal sites and surfaces. Although glucocorticoid treatment of mouse models mimics some
167 of the findings seen with clinical use, the extent to which non-human models mirror the
168 pathophysiology in humans remains unclear.

169 In the following section the individual effects of glucocorticoids on 1, osteoblasts, 2, osteocytes, and
170 3, osteoclasts will be discussed in terms of in vitro and in vivo actions. These sections reflect the
171 majority of recent studies investigating how glucocorticoids affect bone. There is, however, a small
172 number of studies that report consequences possibly mediated by other cells. For example, a
173 preliminary report indicated that mice that lack lymphocytes are protected against glucocorticoid
174 induced changes in bone density, suggesting a possible role for these cells in GIOP.¹⁸

175

176 II.I.I Effects on osteoblasts

177 In vitro effects:

178 In contrast to the clearly detrimental impact that therapeutic glucocorticoids have on bone in the
179 clinical setting in many in vitro situations, glucocorticoids have an important and positive role in the
180 commitment and differentiation of cells of the osteoblast lineage. Glucocorticoids have a stimulatory

role in the differentiation of uncommitted mesenchymal precursor cells to the osteoblastic lineage and high doses of glucocorticoids are generally part of the differentiation medium in protocols for the differentiation of these cells.^{19,20} Glucocorticoids demonstrate clear stimulatory activity on the expression of a range of cellular markers related to osteoblast function, including osteocalcin and alkaline phosphatase.²¹⁻²⁴ Glucocorticoids show inconsistent effects on cellular proliferation but in general high doses of glucocorticoids slows the proliferation rate of mature osteoblast like cells in culture.²⁵ The observation that glucocorticoids in vivo usually result in a dramatic decrease in bone formation but in vitro actions are largely stimulatory has been difficult to explain. It is possible that there is dose dependency with low levels of glucocorticoids being stimulatory and high doses being inhibitory for osteoblasts.²⁶ Other lines of evidence suggest that in vitro effects of glucocorticoids in culture are more complex than previously considered. For example, within a single primary culture of osteoblastic cells there are various different populations present. It has been suggested that more mature osteoblasts have stimulatory paracrine functions on less mature osteoblast precursors and that these are glucocorticoid dependent. For example, disruption of glucocorticoid signalling by the artificial introduction of 11 β -HSD2 into mature osteoblasts results in reduced differentiation of less mature osteoblasts within the same culture, an effect likely due to alterations of expression of wnt or wnt-related genes.²⁷ These results indicate that the communication between various types of bone cells at different stages of differentiation is likely to be complex and glucocorticoids appear important in these communication pathways. A recent review focussing on the various mechanisms by which glucocorticoids affect osteoblast function has been published by Frenkel et al.²⁸

Glucocorticoids influence the proliferation, differentiation or function of osteoblasts but most dramatically they influence their survival and death. It is now clear that osteoblast apoptosis has an important role in bone physiology.²⁹ Glucocorticoids stimulate osteoblast apoptosis in vitro, triggering the rapid activation of the kinases Pyk2 and JNK and increasing reactive oxygen species (ROS) in primary cultures.^{30,31} Glucocorticoids can increase apoptosis via increased endoplasmic reticulum stress and glucocorticoid actions through this pathway synergise with TNF α .³²

207 Glucocorticoids also regulate the expression and activity of pro-apoptotic factors of the Bcl2 family
208 such as Bim.³³ Knockdown of Bim in osteoblasts protects against glucocorticoid induced apoptosis
209 and silencing of E4BP4 attenuates Bim expression and also blocks glucocorticoid induced apoptosis
210 in osteoblasts.³⁴ Glucocorticoids also increase expression of Bak (another Bcl2 family member) and
211 decrease expression of Bcl-XL, a pro-survival Bcl2 protein.³⁵ Dexamethasone can induce Bcl2
212 mediated cell death via induction of p53.³⁶ As such there appears to be multiple pathways by which
213 glucocorticoids induce apoptosis of osteoblasts.

214 A vast range of studies have attempted to identify specific pathways by which glucocorticoids act on
215 osteoblasts in culture. The most prominent targets proposed include: effectors of apoptosis,
216 RANKL/OPG signalling, wnts and their inhibitors, microRNAs, IL-11 and BMP/notch signalling.

217 Glucocorticoids stimulate expression of RANKL and suppress expression of OPG in primary cultures
218 of osteoblasts and osteoblast like cell lines.³⁷⁻³⁹ These changes would be expected to generate a pro-
219 osteoclastogenic signal. The significance of osteoblast expressed RANKL has recently been
220 questioned with the osteocyte now considered to be the most important source of RANKL in normal
221 physiology.^{40,41}

222 Wingless (wnt) signalling is firmly established as a critical mediator of many of the anabolic and
223 catabolic signalling pathways in bone.⁴² Glucocorticoids have dramatic impacts on a range of wnt
224 related genes. At low doses glucocorticoids promote the secretion of wnt9a and wnt10b. At higher
225 doses glucocorticoids suppress intracellular wnt signalling in osteoblasts resulting in a suppression of
226 osteoblast differentiation.^{43,44} Significant interest has focussed on the synthesis of wnt inhibitors
227 such as DKK-1 and sclerostin. Whereas sclerostin will be discussed in the next section since it is
228 expressed exclusively in osteocytes, DKK1 is an important wnt inhibitor which is expressed in
229 osteoblasts and reported to be positively regulated by glucocorticoids.⁴⁵ DKK1 appears to have a
230 negative impact on bone formation but also causes a reduction in the expression of OPG by

osteoblasts,⁴⁶ which would favour an increase in osteoclastogenesis and bone resorption, in addition to the reported suppression of anabolic osteoblast behaviour.

Other osteoblastic signalling pathways targeted by glucocorticoids include insulin-like growth factors, transforming growth factors, basic fibroblast growth factor, and platelet-derived growth factors. In vitro, glucocorticoids suppress the expression of IGF I and PDGF, which possess anabolic mitogenic actions in osteoblasts, whilst reducing the anabolic actions of TGFb.⁴⁷⁻⁴⁹ Novel cellular targets in osteoblasts which appear to be influenced by glucocorticoids in vitro include IL-11⁵⁰, E3 ubiquitin ligases⁵¹ and microRNA-199a.²⁵

239

In vivo effects:

Much of what we know about the impact of glucocorticoid on osteoblast function comes from animal models of GIOP. Although these models appear to recapitulate some of the features seen in human GIOP there is considerable diversity and variability in the phenotypes seen with the models used. This raises questions as to the applicability of these models to the human situation. Mouse models vary in terms of the strain used; the animal age and gender; the glucocorticoid type, route and dose employed; and the skeletal site examined. The C57/B6 mouse strain is increasingly employed as this background is generally most efficient when using tissue targeted transgenic models via the Cre/lox approach. Recent evidence suggests that this mouse strain is relatively resistant to the effects of glucocorticoids on bone compared to other mouse strains.⁵² It is not clear whether this reflects a bone specific difference in glucocorticoid sensitivity or a more generalised disparity. Although this variability between animal strains in terms of glucocorticoid sensitivity and mechanisms of glucocorticoid adverse consequences makes conclusions more difficult to draw, it is possible that what is seen in mice is some of the clinical variability seen in humans. The findings from mice models might need to be considered in their aggregate form rather than depending too much

on individual models. A comprehensive review of the various non-human models used to study GIOP has recently been published.¹⁶ In addition to being useful for the study of GIOP these models have also indicated that glucocorticoid signalling is important for normal mineralisation of vertebral bones and bone growth at some surfaces.

The acceptance of mice as models for human GIOP started with a highly influential study which examined the Swiss Webster mouse strain treated with subcutaneous pellets of prednisolone.⁵³ These studies demonstrated that glucocorticoids could induce osteoblast and osteocyte apoptosis in vivo. The effects of glucocorticoids on osteoblasts appeared to dominate those on osteoclasts and bone resorption. The direct cellular targets of glucocorticoids within bone have been examined in subsequent studies utilising C57/B6 mice. These studies include tissue selective blockade of glucocorticoid signalling in specific cell lineages using the 11 β -HSD2 enzyme or selective deletion of glucocorticoid receptors. A potentially important distinction between these approaches is that models using 11 β -HSD2 will have reduced glucocorticoid signalling through both GR and MR if these receptors are present within target cells whereas selective GR or MR deletion will only target these aspects of glucocorticoid signalling. Another important caveat using these approaches is that it is now clear that the promoters used to drive selective expression within bone have some limitations in that expression in tissues of interest is not normally complete and expression of transgenes can occur to a limited extent in off target tissues, for instance in selective regions of the brain during development.⁵⁴⁻⁵⁶ This off target expression may differ between strains.

11 β -HSD2 is a glucocorticoid inactivating enzyme which has expression primarily in MR expressing tissues such as kidney, colon and salivary gland. There is additionally some expression within the brain and in fetal tissues.^{57,58} Osteoblastic cells (osteoblasts and osteocytes) in the adult mouse do not express 11 β -HSD2 although the enzyme has been detected in fetal bone.⁵⁹ 11 β -HSD2 is highly effective at reducing glucocorticoid signalling and when expressed in osteoblasts appears to entirely block the effect of physiological concentrations of endogenous glucocorticoids.⁶⁰ The enzyme is also

280 effective at blocking the action of prednisolone.⁶¹ As such, transgenic expression of 11 β -HSD2 within
 281 osteoblasts has been utilised to examine the impact of glucocorticoids on these cells. Expression of
 282 11 β -HSD2 within osteoblasts has been reported in two different strains of mice with different
 283 promoters. In the C57/B6 strain, expression of 11 β -HSD2 gene was under the control of the
 284 osteocalcin promoter and as such would be expected to be expressed in mature osteoblasts and
 285 osteocytes.⁶¹ These mice did not have an obvious basal phenotype but were protected against the
 286 actions of glucocorticoids on osteoblast apoptosis and loss of bone density. In the CD1 strain, 11 β -
 287 HSD2 has been driven under the control of the 2.3Kb Col1A1 promoter.^{62,63} This truncated form of the
 288 full type I collagen promoter is expressed in mature osteoblasts and osteocytes but not in other cell
 289 types that normally produce type I collagen.⁶⁴ These mice had a subtle basal phenotype with
 290 reduced bone density of the vertebrae implying an impairment of bone mineralisation.⁶³ These mice
 291 also had delayed ossification of the cranial bones and reduced periosteal circumference of long
 292 bones indicating reduced periosteal apposition of bone.^{65,66} These mice were also protected against
 293 the adverse effects of glucocorticoids on bone, specifically the reduction in bone formation rate and
 294 increase in endosteal bone resorption seen in controls.⁶⁷

295 Mice with targeted deletion of the GR in osteoblastic cells have also been generated. These mice had
 296 Cre driven by the Runx2 promoter which is expressed in cells throughout the osteoblast lineage.⁵⁰
 297 These mice had a basal phenotype characterised by mildly reduced bone size and reduced bone
 298 density. As with the mice above this strongly indicated that endogenous glucocorticoids are not
 299 essential for bone formation but do have a mild anabolic effect on bone. When treated with
 300 prednisolone these mice did not demonstrate the bone loss seen in their wild type equivalents.
 301 These negative effects of glucocorticoids still occurred in mice where the GR was modified such that
 302 it was not able to form dimeric complexes (the dim-dim mice). This implies that the actions of
 303 glucocorticoids on osteoblasts are mediated primarily by the monomeric form of the receptor (a
 304 mechanism associated with transrepression and typically associated with anti-inflammatory actions)
 305 rather than the dimeric form traditionally thought of as mediating the beneficial metabolic actions of

306 glucocorticoids. This study identified suppression of IL-11 as an important mediator of the adverse
307 effects of glucocorticoids on bone.

Comment [RSH4]: Is it worth having a table summarising the GC targeted inhibition in OBs and reporting the phenotype?

Yes definitely

308 Although it has been generally assumed that the GR is the most important target of glucocorticoids
309 in the osteoblast it has been reported that the MR is also expressed in these cells.⁶⁸ In mouse models
310 blockade of MR signalling either through the use of the MR antagonist spironolactone or through
311 transgenic deletion of the MR results in some protection against the effects of therapeutic
312 glucocorticoids.⁶⁹ The relative importance of GR and MR signalling in bone and whether there are
313 interactions between the GR and MR signalling pathways has not been determined.

314

315 II.I.II Effects on osteocytes:

316 Our understanding of the role that osteocytes play in the coordination of bone remodelling has
317 developed rapidly over the last two decades. A role for osteocytes in the development of GIOP was
318 suggested at an early stage and in particular a role for osteocyte apoptosis was demonstrated in
319 animal models.⁵³ Thus, osteocyte appears to be extremely sensitive to glucocorticoids.

320

321 Evidence for osteocyte apoptosis.

322 Evidence for a role of glucocorticoids in osteocyte function initially came from animal models of
323 glucocorticoid treatment in which apoptosis of osteocytes could be demonstrated.^{53,70} These
324 observations were supported by studies examining human bone from individuals that had been
325 exposed to high levels of glucocorticoids where signs of osteocyte apoptosis were also seen.^{53,71}
326 Osteocytes are thought to be long-lived cells and it is uncertain whether osteocytes that have
327 apoptosed can be replaced by new osteocytes. As such death of osteocytes would be likely to have
328 prolonged consequences for the organism. Apoptosis of osteocytes was also demonstrated in mice

329 treated with a high dose of prednisolone (2.4mg/kg/d over 28 days) while osteocytes remained
330 unaffected at a lower dose of 1.4mg/kg/d, suggesting that there may be a threshold for the
331 development of osteocyte apoptosis.⁷²

332 Various factors have been found to protect against glucocorticoid induced apoptosis of osteocytes in
333 animal models. These include PTH, bisphosphonates, calcitonin and OPG.⁷³⁻⁷⁶ Whether this
334 mechanism contributes to the therapeutic efficacy of some of these agents in human GIOP is unclear
335 and difficult to test clinically.

336 It should be noted that osteocyte apoptosis in GIOP has not been a universal finding. Indeed, there
337 was no evidence of osteoblast or osteocyte apoptosis in control mice treated with glucocorticoids in
338 the study examining the effects of osteoblast/osteocyte specific deletion of GR on the sensitivity of
339 bone to glucocorticoids.⁵⁰ This lack of osteocyte apoptosis was manifest despite glucocorticoids
340 having a clearly detrimental effect on bone formation and bone strength. It is possible that this
341 observation reflects differences between strains and glucocorticoid dosing but it also implies that
342 glucocorticoid induced apoptosis of osteocytes is not an essential step for glucocorticoids to have
343 their negative effect on bone. In this context there may be a parallel with the clinical situations of
344 osteoporosis and osteonecrosis. Therapeutic glucocorticoid administration can cause both but the
345 development of clinically significant osteonecrosis is generally much rarer than that of osteoporosis.

346

347 Glucocorticoid treatment has been demonstrated to induce significant structural changes in the
348 environment of the osteocyte. Glucocorticoids adversely affect fluid flow in the canalicular network.
349 ⁷⁷ This effect would be expected to have detrimental effects on osteocyte health but could also
350 directly influence bone mechanical strength through effects on bone tissue hydration. Glucocorticoid
351 treatment is also associated with an increase in mean osteocyte lacunar size.⁷⁸ There is, in addition,
352 a reduction in mineralisation in the bone adjacent to the osteocytes, a phenomenon referred to as

353 'osteocytic osteolysis'.⁷⁸ This suggests that part of the anatomical pathology involved GIOP could be
 354 microscopic changes to bone mineral properties through periacinar osteolysis or
 355 hypomineralisation. The mechanism by which glucocorticoids cause these changes is not
 356 established. These changes could explain the relatively rapid change in fracture risk during
 357 glucocorticoid treatment and its reversibility. In addition, this type of microarchitectural change
 358 would reduce bone strength disproportionately to the change in BMD measured by DXA, thus
 359 accounting for the increased fracture risk observed for the same level of BMD in GIOP.

360 The group that described the microscopic changes in osteocyte lacunae discussed above have
 361 attempted to define the cellular processes that are responsible for these changes. They found that
 362 glucocorticoid treatment was associated with expression of a range of genes in osteocytes
 363 associated with autophagy.^{72,79} Autophagy is a cellular pathway designed to maintain cellular
 364 homeostasis by degrading damaged organelles through formation of autophagosomes. Osteocytes
 365 are reported to respond to glucocorticoid treatment with an increase in autophagy markers and the
 366 accumulation of autophagosome vacuoles. It was hypothesised that autophagy maintained cell
 367 viability in the presence of glucocorticoids.⁷⁹ The balance between protective autophagy clearance of
 368 damaged organelles and destruction of key cellular components may shift across tissue sites and
 369 with different glucocorticoid doses making its contribution to glucocorticoid mediated suppression
 370 of osteoblasts in vivo difficult to truly appreciate. Piemontese et al. tested whether genetic
 371 suppression of autophagy was associated with increased sensitivity of osteocytes to
 372 glucocorticoids.⁸⁰ They deleted autophagy related gene 7 (Atg7), a gene central to the autophagy
 373 process, from osteocytes using the Dmp1Cre promoter. In control mice glucocorticoids stimulated
 374 autophagy in osteocytes and this was blocked in transgenic mice. However, there was no impact of
 375 autophagy suppression on the effects of glucocorticoids on bone. Interestingly, chemical inhibitors
 376 of autophagy have demonstrated protection against glucocorticoid induced bone loss and
 377 maintained bone formation.⁸¹ However, autophagy had now been reported to occur in osteoclasts
 378 exposed to glucocorticoids.^{82,83} Selective deletion of Atg7 in osteoclast precursors suppressed

379 glucocorticoid induced increases in bone resorption and bone loss in mice without any impact on
380 osteoblast differentiation.⁸² Currently it appears that glucocorticoids induce autophagy in both
381 osteocytes and osteoclasts but that the process in osteoclasts but not osteocytes impacts on bone
382 strength.

383 The osteocyte is now clearly established as being central to the process of bone remodelling through
384 secretion of several key regulators of bone physiology.⁸⁴ Osteocytes can generate OPG and RANKL.
385 Glucocorticoids down regulate the production of OPG in osteocytes whereas the expression of
386 RANKL appears unchanged.^{85,86} Osteocyte secretion of RANKL appears to be a requirement for loss
387 of cortical bone in mice treated with glucocorticoids.⁸⁶ In this study glucocorticoids did not directly
388 regulate RANKL in osteocytes but rather reduced the expression of OPG which allowed greater
389 activity of the RANKL present. The osteocyte is also an important producer of wnt signalling
390 antagonists such as sclerostin and DKK1. Glucocorticoids appear to increase the production of both
391 sclerostin and DKK1 by osteocytes.⁵¹ The role of sclerostin in glucocorticoid induced bone loss has
392 been examined in studies using anti-sclerostin antibodies and animals with genetic knockout of
393 sclerostin. Treatment of mice with anti-sclerostin antibodies prevented the glucocorticoid induced
394 reduction in bone formation seen with placebo treated mice.⁸⁷ One study reported that these
395 antibodies protected mice from glucocorticoid induced osteocyte apoptosis.⁸⁸ A study using
396 sclerostin/Sost knockout mice found that sclerostin deficiency protected against glucocorticoid
397 induced bone loss but did not protect against a decrease in bone formation or an increase in
398 osteoblast/osteocyte apoptosis.⁸⁵ The protection appeared due to preservation of OPG levels and a
399 protection against increased bone resorption that was seen in wild type mice.⁸⁵

400 These studies suggest that all of these molecules may have a role in different aspects of the effects
401 of glucocorticoids depending on the model used. With regards to sclerostin and DKK1, it is currently
402 unclear which particular pathway is most relevant in animals and humans. In particular it is not clear
403 whether they both have essential roles or if there is compensation or redundancy between them.

404

405 Controversies in the field.

406 A major issue is that osteocyte apoptosis should leave long standing consequences on bone since
407 these cells are thought to be long lived. However, epidemiological studies indicate that the increased
408 risk of fracture during treatment with glucocorticoids declines rapidly when the treatment is
409 discontinued. It is possible that in humans there is a spectrum of osteocytic damage that can
410 manifest as osteoporosis if the degree of osteocyte damage is modest, but as frank osteonecrosis if
411 the degree of osteocyte damage is more extensive. It is also not clear why some studies in mice do
412 not show any evidence of osteocyte apoptosis even when there are clearly negative effects of
413 glucocorticoid treatment on other aspects of bone health.

414 A further limitation is that the transgenic models discussed above which target glucocorticoid
415 receptor signalling in osteoblasts also disrupt glucocorticoid signalling in osteocytes. To date, no
416 osteocyte or osteoblast specific GR deletion model has been produced and evaluated in the context
417 of GIOP. As such the relative contributions of osteoblasts and osteocytes to the observed
418 phenotypes are not clear. It is possible that there may be independent contributions from both of
419 these cell types which require a fuller exploration.

420

421 II.I.III Effects on osteoclasts:

422 The effects of glucocorticoids on osteoclasts have been examined in vitro and in vivo with indirect
423 inferences being made through clinical studies. The examination of the role of osteoclasts in GIOP
424 has been complicated since glucocorticoids appear to have direct effects on osteoclasts or their
425 precursors but also have powerful indirect influences on osteoclastogenesis and osteoclast function
426 via effects on osteoblasts and osteocytes.

427 In vitro studies have shed light on the direct actions of glucocorticoids in human osteoclasts where
 428 they increase resorption activity and pit formation.^{89,90} High doses of glucocorticoids are used in
 429 culture media to promote the growth and differentiation of osteoclasts.⁹¹ Greater mechanistic
 430 insights into these observations have come from murine osteoclast culture studies where addition of
 431 glucocorticoids prolongs longevity through their activation of the GR receptor.^{92,93} However, these
 432 same studies have identified that therapeutic glucocorticoids are also able to suppress osteoclast
 433 differentiation and activation in vitro by increasing apoptosis and interfering with cytoskeletal
 434 reorganisation and rendering them less responsive to the pro-osteoclastogenic actions of M-CSF.^{92,93}
 435 Similarly, osteoclast activity is suppressed within cultures of osteoclast in rats as a result of increased
 436 apoptosis in response to glucocorticoids.⁹⁴ Overall, similar to what has been found in osteoblasts,
 437 the effects of glucocorticoids on osteoclast formation and bone resorbing capacity appear to be dose
 438 dependent with mostly stimulatory actions at low concentrations and inhibitory effects at very high
 439 concentrations.

440 The effects of glucocorticoids on osteoclasts in vivo have been examined in murine models of
 441 glucocorticoid excess.^{93,95,96} Bone resorption/osteoclast activity is increased during early treatment
 442 with glucocorticoids supporting in vitro observations that glucocorticoids increase the survival of
 443 osteoclasts. Targeted abrogation of glucocorticoid signalling in osteoclasts using 11 β -HSD2
 444 expression resulted in protection against this initial increase in osteoclast activity in mice treated
 445 with prednisolone.⁹³ However, with prolonged exposure to high levels of glucocorticoids the number
 446 of osteoclasts is reduced due to a delay in the differentiation of new osteoclasts.⁹²

447 The most provocative studies in this area examined the deletion of the GR in osteoclasts using the
 448 LysM^{CRE} transgene.⁹² LysM^{CRE} is expressed in cells of the monocyte/macrophage lineage including
 449 osteoclasts. The mouse strain had a mixed 129/C57 genetic background. Rather than generating an
 450 osteoclastic phenotype the main consequence of osteoclast GR deletion was unexpectedly protected
 451 against the fall in bone formation during treatment with dexamethasone (10mg/kg daily injections)

452 as assessed by mineral apposition rate and serum osteocalcin levels. The osteoblasts did not appear
453 to be protected against glucocorticoid-induced apoptosis. This study implies that at least some of
454 the effects of glucocorticoids on the osteoblast might be mediated via the osteoclast. No mechanism
455 for such communication was identified. Although not examined in this context several established
456 signalling pathways by which osteoclasts can potentially suppress bone formation have been
457 reported^{97,98} giving these findings plausibility despite their sharp contrast with most of the existing
458 literature.

459 Similar studies using osteoclast GR deletion have failed to show the same effect. Prednisolone
460 treatment of another mouse (Balb/c background) with GR knockout using the LysM^{CRE} transgene did
461 not demonstrate any protection against the reduction in bone formation as assessed by bone
462 formation rate.⁵⁰ Likewise, the expression of 11 β -HSD2 within osteoclasts using the tartrate resistant
463 acid phosphatase (TRAP) promoter in the FVB/N mouse strain failed to protect mice against a
464 decrease in bone formation as assessed by serum osteocalcin levels in response to treatment with
465 slow release prednisolone pellets.⁹³ It is possible that these differences relate to subtle differences in
466 strain, glucocorticoid dose or experimental set up. For instance the data regarding the effect of
467 osteoclasts on bone formation examined the growth of the calvarial bone surface.⁹² As discussed
468 earlier the outer cortex of bone seems to respond differently to glucocorticoids⁶⁷ and thus the
469 choice of surface may be an important factor in these results. Overall, given that there is only one
470 study in support, on the current balance of evidence a significant role for osteoclasts in
471 glucocorticoid induced suppression of bone formation throughout the skeleton appears unlikely.

472

473 Summary:

474 Glucocorticoids have multiple effects on osteoblasts, osteocytes and osteoclasts (summarised in
475 figure...). Many of these effects appear to be individually very powerful in determining specific

Comment [M5]: Need to add figure.

phenotypes when examined in mouse models and this implies that no single mechanism is likely to mediate all of the effects seen in the clinical setting. Reduced bone formation at trabecular bone sites and increased endocortical resorption appear to be the most consistent pathological findings. The results appear to indicate that the osteocyte is the most important target of glucocorticoids but several major signalling pathways and cellular processes are all affected simultaneously. It should also be noted that none of the studies described above examined the effects of glucocorticoids in the context of inflammation. Clinical studies of patients treated with therapeutic glucocorticoids for various conditions consistently demonstrate that inflammation and the activity of the underlying disease being treated can have a substantial effect on bone independent of glucocorticoid use or more likely through complex interactions between glucocorticoids, the underlying illness and bone metabolism.

487

II.II Other endocrine and non-endocrine effects on bone

Glucocorticoids have effects on bone independent of their direct actions on bone cells. These effects are however difficult to study in animal models, particularly those that do not simulate an underlying disease being treated. Therapeutic glucocorticoids are well known to reduce sex steroid levels and this could have an adverse impact on bone.⁹⁹ The reduction in sex steroid levels is likely to be greater in people with serious inflammatory illness which in itself is likely to impact on the hypothalamo-pituitary-gonadal axis.¹⁰⁰ Evidence in support of this notion comes from clinical trial data which indicate that premenopausal women are relatively protected against the effects of glucocorticoids on fracture risk.¹⁰¹ Clinical studies have indicated that estrogen treatment of post-menopausal women^{102,103} or testosterone (but not nandrolone) treatment of men¹⁰⁴ taking glucocorticoids results in an increase in spine but not hip bone density (as measured by DXA). No fracture data are available. In women taking glucocorticoids who are already taking HRT there does not appear to be

500 any increase in bone density with continued use whereas the addition of intermittent PTH injection
501 substantially improves BMD at the spine.¹⁰⁵

502 Glucocorticoids also have complex effects on calcium, vitamin D metabolism and parathyroid
503 hormone. The literature relating to these actions is relatively old but indicates that glucocorticoids
504 interfere with intestinal calcium absorption and increases renal calcium excretion.¹⁰⁶ Early research
505 also suggested a role for altered parathyroid hormone levels in the pathogenesis of GIOP.¹⁰⁷
506 However, a comprehensive review failed to find strong evidence for a role of parathyroid hormone
507 in the detrimental effects of glucocorticoids on bone.¹⁰⁸

508 The effects of glucocorticoids are also attributable to changes in other circulating or locally produced
509 hormones. The GH/IGF1 axis is known to have anabolic effects on bone growth and bone density.¹⁰⁹
510 These hormones are suppressed by high levels of glucocorticoids. Many in vitro studies have
511 indicated that the suppressive effects of glucocorticoids on osteoblast function can be partially
512 reversed by GH and/or IGF1 treatment.¹⁰⁹ However, there are very few clinical studies examining this
513 issue. Small studies in which children taking glucocorticoids for inflammatory bowel disease or
514 arthritis were treated with GH indicated that GH therapy could improve some measures of bone
515 formation and reverse effects of glucocorticoids on growth but these studies lacked control
516 groups.^{110,111}

517 Glucocorticoids also have adverse effects on muscle strength, which known to influence bone
518 strength through mechanical loading¹¹². This association of glucocorticoids with muscle strength is
519 well characterised in Cushing's disease where proximal myopathy is a characteristic and relatively
520 specific feature of glucocorticoid excess. These actions are mediated through inhibition of
521 myogenesis and increased proteolysis and atrophy of muscle fibres.¹¹³⁻¹¹⁵ As a consequence,
522 glucocorticoid treatment appears to be a risk factor for falls. However, as discussed elsewhere, in a
523 disease situation it is very difficult to disentangle the effect of glucocorticoid treatment from that of
524 the underlying disease. Indeed, in certain inflammatory myopathies including polymyositis and

dermatomyositis the application of therapeutic glucocorticoids protects against muscle wasting through the suppression of disease activity.¹¹⁶ Some evidence supporting a positive role of endogenous glucocorticoids in maintaining muscle mass during inflammatory disease comes from mice in which 11 β -HSD1 expression in muscle has been deleted.¹¹⁷ These mice have normal muscle size and characteristics in the basal state but in response to inflammation, muscle loss is much greater in 11 β -HSD1 deficient mice. These data highlight the clinical dilemmas in treating inflammatory muscle and joint diseases with glucocorticoids, where such treatment may result in detrimental, neutral or strongly positive effects on muscle strength and falls risk, depending on the impact of glucocorticoids on the underlying illness.

534

535 II.III Metabolic consequences mediated through bone cells

In addition to the deleterious effects of therapeutic glucocorticoids on bone, these medications are also associated with an increased risk of impaired glucose tolerance, diabetes or, in people with pre-existing diabetes, worsening of diabetic control.^{118,119} These effects have previously been assumed to be due to actions of glucocorticoids on tissues classically associated with insulin secretion or sensitivity such as the liver, muscle and pancreas.¹¹⁸ However, recent studies suggest that there may in addition be a role for bone in the development of dysmetabolism associated with glucocorticoid treatment¹²⁰.

Multiple studies in mice indicate that osteocalcin has metabolic effects. In particular osteocalcin, and in particular the uncarboxylated form of osteocalcin, appear to improve glycemic control and insulin sensitivity through effects on insulin secretion and insulin sensitivity.¹²¹ Cross-sectional studies in humans also demonstrate correlations between serum undercarboxylated osteocalcin and diabetes risk.¹²² The exact molecular pathways affected are unclear particularly since the identity of the

548 osteocalcin receptor(s) is still uncertain, although some candidates have been proposed such as the
549 GPRC6A receptor.¹²³

550 Given that the level of osteocalcin in the circulation is dramatically reduced by therapeutic
551 glucocorticoids it was hypothesised that some of the effects of glucocorticoids on systemic
552 metabolism might be mediated by the glucocorticoid induced reduction of circulating osteocalcin
553 concentrations. Studies using 11 β -HSD2 transgenic mice which, as discussed above, selectively
554 express 11 β -HSD2 in osteoblasts and osteocytes, demonstrated that the glucocorticoid induced
555 reduction in serum osteocalcin levels was substantially reduced when glucocorticoid-signalling in
556 osteoblasts was disrupted. Furthermore, these mice had preserved glucose tolerance compared to
557 littermate control mice that did not have 11 β -HSD2 expression in bone.¹²¹ This protection against
558 the effects of glucocorticoids on glucose tolerance was also seen in wild type mice in which
559 osteocalcin was heterotopically and constitutively expressed in the liver. This rescuing of the
560 dysglycemic phenotype was seen with heterotopic expression of either wild type osteocalcin (which
561 would be expected to be carboxylated in vivo) or a mutant form of osteocalcin which lacked the
562 ability to be carboxylated. It is unclear whether similar protection might exist in humans.

563 An alternative approach has been to examine any possible impact of bone active treatments in
564 patients treated with glucocorticoids. It is known that bisphosphonates reduce, and teriparatide
565 stimulates osteocalcin synthesis and as such these treatments might result in differences in glycemic
566 control when used in the treatment of GIOP. One small prospective study involving 111 people
567 taking glucocorticoids that were treated with either bisphosphonates or teriparatide reported a
568 small but significant decrease in HbA1c in people who took teriparatide whereas there was no
569 change in HbA1c in those that took bisphosphonates or just calcium and vitamin D.¹²⁴

570 Although osteocalcin has been the most studied mediator of effects of bone on systemic metabolism
571 it is likely that other pathways exist. These pathways have been reviewed elsewhere but have not
572 yet been examined in the context of glucocorticoids and bone.^{125,126}

573 Given that there is a role for excess glucocorticoids in the development of systemic dysmetabolism
574 there is a possibility that endogenous glucocorticoids have a similar influence on systemic
575 metabolism via an action on bone. Circumstantial evidence for this exists in that transgenic deletion
576 of 11 β -HSD1 globally protects mice against the adverse effects of glucocorticoids on energy
577 metabolism.¹²⁷ However, deletion of 11 β -HSD1 in classical target tissues of glucocorticoids such as
578 liver, fat or muscle failed to prevent these effects suggesting that other tissues also contribute to the
579 adverse metabolic phenotype seen with glucocorticoid exposure.

580

581 **III Endogenous glucocorticoids and bone**

582 In this section we will review data relating to the effects of endogenous glucocorticoid excess on
583 bone. We will review the bone phenotype in Cushing's disease but also more subtle states of
584 autonomous states of circulating glucocorticoid excess. We will then examine the role of tissue
585 specific changes in glucocorticoid action focussing primarily on the role of 11 β -hydroxysteroid
586 dehydrogenase enzymes.

587

588 III.I States of circulating glucocorticoid excess, Cushing's disease and autonomous cortisol
589 production

590 III.I.I Bone disease in Cushing's disease/syndrome.

591 As discussed in section II.1, there is strong evidence that endogenous glucocorticoids are required
592 for normal bone metabolism and osteoblastogenesis.^{63,128} In contrast, in Cushing's disease and other
593 clear cut forms of endogenous circulating glucocorticoid excess, there is normally a substantial
594 negative impact on bone (reviewed in Toth and Grossman¹²⁹). This was recognised early on by
595 Cushing, and bone related complications of Cushing's disease are clearly evident in clinical practice.¹
596 More recent studies that have attempted to quantify the bone effects of endogenous Cushing's
597 syndrome have generally been small (up to around 180 patients) and varied according to the
598 number of patients with each underlying cause of Cushing's (pituitary, adrenal, ectopic,
599 adrenocortical cancer etc.), and length of time before diagnosis was made. Despite this diversity, the
600 studies have been very consistent in indicating a substantially increased risk of fracture (typically a
601 fracture prevalence of 50% is reported) and a greater chance of having very low bone density (below
602 a T-score of -2.5) when assessed by DXA. The incidence and prevalence figures depend on the extent
603 to which fractures are searched for. In a self-report survey of 125 patients with endogenous
604 Cushing's syndrome and age and sex matched controls, fracture risk appeared to be elevated

605 substantially in the 2 years prior to diagnosis with an incidence rate ratio of 6 in patients with
606 Cushing's syndrome.¹³⁰ Interestingly there was no evidence of an increased risk of fracture prior to 2
607 years before the diagnosis. Additionally, after successful treatment the reported fracture rate was
608 also no different from that of controls. Although an important study with a high (83%) response rate,
609 the data was limited by the study's focus on clinical fractures.

610 This type of analysis cannot accurately determine the risk of vertebral fractures. Vertebral fractures
611 are frequently misdiagnosed or missed. As a consequence, the overall rate of vertebral fractures of
612 any origin is usually grossly underestimated unless examined for specifically.¹³¹ Vertebral fractures,
613 even if asymptomatic, are amongst the strongest risk factors for further fracture and premature
614 mortality.^{131,132} The standard approach to the diagnosis of vertebral fractures is to examine for loss
615 of vertebral height on spine radiographs using the Genant classification (with a fracture defined as a
616 loss of anterior vertebral height of 20% or more)¹³³. A study by Tauchmanova et al. focussed
617 particularly on the risk of spine fractures in patients with endogenous Cushing's syndrome of various
618 etiologies and examined spine radiographs in cases and controls.¹³⁴ In an analysis of 80 patients and
619 80 controls, vertebral fractures were present in a remarkable 76% of patients with Cushing's. In an
620 equally remarkable 85% of patients with a vertebral fracture, multiple fractures were present. Only
621 24% of spine fractures were known to the patient.

622 A comprehensive and contemporary analysis of bone disease in a cohort of patients with
623 endogenous Cushing's syndrome was reported by Belaya et al.¹³⁵ All patients had chest radiographs
624 and AP and lateral spine radiographs. In 182 patients studied, 81 patients had fractures. 70 of these
625 patients had fractures of the spine. 53 out of these 70 patients had multiple vertebral fractures. Out
626 of over 150 fractures just 7 were non rib, non-vertebral fractures. These figures indicate that
627 prevention of spine fractures should be the major skeletal priority in patients with endogenous
628 Cushing's syndrome.

629 Although Cushing's syndrome is associated with bone loss (as assessed by DXA) and osteoporotic
630 fractures, the utility of bone mineral density scans in predicting fracture is limited. In the studies
631 described above, fractures (and in particular spine fractures) occurred in some patients with
632 relatively well preserved BMD.¹³⁴ In the largest study, bone density measured by DXA was not
633 predictive of fracture in a multivariable model that took into account the severity of
634 hypercortisolaemia.¹³⁵ The only predictor of fracture in this study was the severity of Cushing's.
635 However in an earlier study spine BMD was a predictor of vertebral fracture in Cushing's.¹³⁴ Since the
636 severity of Cushing's is associated with reductions in bone mass it is likely that the severity of
637 Cushing's and the decrease in measured BMD both provide clinically useful information in the
638 assessment of fracture risk in these patients. Trabecular bone score (TBS; a non-invasive measure of
639 trabecular bone architecture derived from spine DXA scans) has also been evaluated in patients with
640 Cushing's.¹³⁵ Values were found to be significantly reduced (indicating impaired trabecular bone
641 structure) but the scores did not have predictive value in estimating the risk of vertebral bone
642 fracture. Advanced imaging techniques such as high resolution peripheral quantitative CT (HR-pQCT)
643 and hrQCT of vertebral bone, and techniques for in vivo examination of material properties such as
644 microindentation, which have all been used in patients treated with glucocorticoids, have not yet
645 been reported in patients with Cushing's.

646 Other potential predictors of fracture have also been examined in Cushing's. In one study fracture
647 risk at the spine appeared to be independent of the presence of menstrual irregularities with
648 amenorrheic women having a similar risk of fracture and BMD to those with eumenorrhea.¹³⁴
649 Another study however suggested that reduction in BMD was more likely in women with estrogen
650 deficiency.¹³⁶ Fracture risk was higher in patients with ectopic ACTH syndrome, presumably as a
651 result of the higher cortisol levels usually found in this condition.¹³⁴ Serum osteocalcin levels have
652 also been associated with fracture risk but again this relationship appears to be mediated by the
653 levels of cortisol present.¹³⁵ In terms of prediction of changes in BMD, the correlation between the
654 extent of reduction in BMD and degree of cortisol excess has been reported in eumenorrheic women

with Cushing's.¹³⁷ The extent of reduction of BMD in patients with Cushing's has also been associated with the duration of disease.¹³⁶

Whereas fracture risk in Cushing's has been quantified in only a small number of studies, changes in biochemical markers of bone turnover have been assessed in at least 16 reports (reviewed in¹²⁹). A finding in all but one of these studies is that serum osteocalcin levels are considerably decreased in Cushing's. The results for other formation markers (PINP, PICP, alkaline phosphatase) show less, if any, change. Bone resorption markers do not appear to change in a consistent fashion in Cushing's. The sensitivity of osteocalcin expression to glucocorticoids is well known and in this situation serum osteocalcin levels might be viewed as a marker of bone tissue glucocorticoid exposure rather than a true bone formation marker. The relationship of low serum osteocalcin with excessive cortisol levels is so strong that serum osteocalcin has been proposed as a diagnostic marker of Cushing's syndrome.¹³⁸ In a group of patients with Cushing's syndrome serum osteocalcin levels were found to be highly correlated with serum cortisol measured at 0800 hrs, 2400 hrs and after a low dose dexamethasone suppression test. In a follow up study, the diagnostic utility of serum osteocalcin in patients presenting with obesity and risk factors for Cushing's syndrome was evaluated.¹³⁹ It was found that osteocalcin had a sensitivity of 74% and a specificity of 97% for the identification of Cushing's syndrome. Additional prospective studies will be required to fully evaluate the clinical utility of osteocalcin as a diagnostic tool in Cushing's syndrome.

The changes in bone status in response to successful therapy have also been evaluated. In the self-report survey of patients with Cushing's syndrome described above the risk of fracture did not appear to be elevated after treatment.¹³⁰ This study was likely to have modest sensitivity in terms of fracture detection given the relatively small number of patients available and the lack of detailed analysis of spine fractures. Studies consistently report a rise in BMD after successful treatment.¹⁴⁰⁻¹⁴³ Although pre-disease BMD is clearly not available in the majority of people it is reported that the deficit in bone mass is largely reversible, at least in younger patients.¹⁴² These changes appear

relatively complex and vary between skeletal sites. Successful treatment is associated with an improvement in spine areal BMD but also an increase in bone area.¹⁴⁰ This suggests the possibility of new bone being laid down on the outside of the vertebral bones (periosteal apposition) when glucocorticoid levels are restored to normal. Intriguingly, after successful treatment bone density and bone area at the wrist were actually reported to decrease.¹⁴⁰ Although the authors proposed that this reflects a redistribution of bone from the appendicular to the axial skeleton it is unclear how such redistribution might occur, particular in relation to bone area, as this would require removal of bone from the outer cortex of the bone. The results are in keeping with the findings in mice that formation of bone at the outer cortex of some bones of the peripheral skeleton is actually stimulated by glucocorticoids rather than being suppressed.⁶⁶ Serum osteocalcin also increases rapidly after treatment.¹³⁸ Whereas there is no correlation between serum osteocalcin and other bone markers prior to treatment, shortly after successful treatment a strong correlation between osteocalcin and bone resorption markers develops (as is normally seen in populations of healthy people).¹³⁸ As such, serum osteocalcin levels appear to primarily reflect cortisol levels in patients with Cushing's prior to treatment, in treated patients they behave more like a traditional marker of bone formation. Although the data in general suggest a reversal of bone disease in patients successfully cured caution should be taken if patients need long term glucocorticoid replacement after cure. In a group of patients successfully cured the continuing use of glucocorticoid replacement was associated with reductions in BMD, BMC and osteocalcin compared to matched controls.¹³⁶ These effects were most evident in women with coexisting estrogen deficiency. This exaggerated sensitivity of estrogen deficient women is in keeping with the greater risk of fracture of post-menopausal women treated with therapeutic glucocorticoids (discussed in section IV.1). As such, glucocorticoid replacement must be particularly carefully monitored in this group.

III.I.II Bone disease is autonomous cortisol secretion.

705 More subtle states of glucocorticoid excess also appear to detrimentally impact on bone. Most
 706 attention has focussed on the concept of subclinical endogenous hypercortisolism, also referred to
 707 as subclinical Cushing's and more recently autonomous cortisol secretion.^{144,145} This condition is in
 708 principle defined by abnormal cortisol secretion in the absence of clinical features of glucocorticoid
 709 excess. It is usually associated with nodules of the adrenal cortex (adrenal incidentaloma, AI). The
 710 condition is controversial and the best diagnostic criteria have yet to be established. In various
 711 studies the criteria differ but the most common component of the diagnosis is failure to suppress
 712 serum cortisol after a 1mg dexamethasone suppression test (DST).¹⁴⁴ Depending on the definition
 713 the condition appears to be relatively common and is driven by the background prevalence of AIs.
 714 The prevalence of AIs based on radiographic series depends heavily on age but it is estimated that
 715 3% of people aged 50 have an adrenal nodule whereas up to 10% of elderly individuals may have
 716 AIs.¹⁴⁴ It has been estimated that up to 30% of patients with AIs have some degree of autonomous
 717 cortisol secretion⁷ and as such up to 1-3% of the population aged 50 and above might have
 718 autonomous cortisol secretion.

719 The research examining the relationship between bone health and the presence of autonomous
 720 cortisol secretion (usually in the context of patients known to have AI) is dominated by the studies of
 721 Chiodini and colleagues. These include cross-sectional, longitudinal, retrospective and prospective
 722 studies examining bone density, bone markers and fracture prevalence and incidence in these
 723 individuals. Most studies reported a reduction in BMD at the spine as assessed by either DXA^{146,147} or
 724 qCT.^{148,149} Trabecular bone score has also been reported to be lower and to predict the development
 725 of fracture in this group of patients.¹⁴⁷ As with bone changes in Cushing's disease the data relating to
 726 the change in BMD at the hip is less clear with some studies indicating a reduction in BMD and some
 727 no change. Differences between studies are likely due to the relatively small number of patients
 728 examined in most studies and heterogeneity in the proportions of men, pre-menopausal and post-
 729 menopausal women. Again, in a similar fashion to that seen with endogenous Cushing's syndrome,
 730 autonomous cortisol secretion is reported to be associated with a decrease in blood osteocalcin

731 levels but no consistent changes in other markers or bone formation or markers of bone resorption.
732 These studies were however relatively small with typically less than 50 patients.

733 The most dramatic findings in these studies are the presence of vertebral fractures. A recent meta-
734 analysis of these studies found that the prevalence of radiographically identified vertebral fractures
735 was 63.6% (CI 56-71%) in patients with autonomous cortisol secretion compared to a prevalence of
736 16% (CI 5-28) in controls.⁷ Interestingly, patients known to have AI that do not meet the criteria for
737 autonomous cortisol secretion were reported to have a higher prevalence of spine fractures (28%)
738 than controls without AI (20-35). This suggested to the authors that some patients with AI with
739 excessive production of cortisol might not be detected by current tests and by implication that all AIs
740 are a risk factor for fracture. The authors of the meta-analysis could not identify any patient related
741 factors that predicted the development of fractures beyond the presence of autonomous cortisol
742 secretion. A study of 570 patients with AIs attempted to determine the threshold cortisol level post
743 1mg DST (using an Abbott TDxFLs cortisol assay) which is best able to predict the presence and the
744 future development of vertebral fractures.¹⁵⁰ It was found that a post DST cortisol level of greater
745 than 2.0 microgram per decilitre (55 nmol/L) was the best criteria in both situations with sensitivities
746 and specificities between 68 and 80%. The presence of cortisol levels above this threshold was
747 associated with an odds ratio of fracture of over 10.

748 These prevalence and incidence rates of vertebral fracture in people with AI are extremely high and
749 could represent a large burden of disease that is currently not being addressed. However, the
750 proportion of these fractures that actually cause symptoms or otherwise impact on patient well-
751 being is unknown. Although difficult to perform, future trials would ideally aim to determine
752 whether AIs (with or without autonomous cortisol secretion) are associated with a greater risk of
753 clinical vertebral fractures, height loss, kyphosis development or reduced quality of life relating to
754 musculoskeletal health. An alternative way of assessing whether autonomous cortisol secretion
755 relating to AIs is associated with clinically significant vertebral fracture is to examine the prevalence

756 of these abnormalities in patients presenting with clinical vertebral fracture. In one study 7 out of 65
757 patients presenting with osteoporosis and spine fracture were found to have subclinical
758 hypercortisolism.¹⁵¹ In a subsequent study of over 600 patients with osteoporosis and no apparent
759 cause the rate of subclinical hypercortisolemia was significantly lower at 1.3%.¹⁵² These patients
760 however had a relatively low rate of reported fracture and in particular of clinical vertebral fracture.
761 On the basis of what is known about endogenous Cushing's and subclinical Cushing's it is reasonable
762 to assume that the development of a vertebral fracture rather than just a low BMD by DXA would be
763 a more sensitive indicator of the presence of abnormal cortisol secretion.

764 Remaining questions in this area include the most appropriate treatment approach to a patient with
765 bone disease related to autonomous cortisol secretion by AI and when and how to investigate for
766 the presence of autonomous cortisol secretion in patients presenting with bone disease. A recent
767 study suggested that adrenalectomy was effective at reducing the risk of new vertebral fracture over
768 a follow up period of 28-40 months.¹⁵³ This study was limited by a lack of randomisation. In the
769 absence of randomised clinical trials it would be reasonable to consider the option of adrenalectomy
770 in patients with AI, autonomous cortisol secretion and bone disease, particularly in the presence of
771 other conditions that might be exacerbated by cortisol excess such as hypertension and diabetes. An
772 additional option is the use of medications that are proven to be effective in the treatment of
773 idiopathic osteoporosis or GIOP associated with therapeutic glucocorticoid use. There is additionally
774 data based on a small number of people that indicates that the bisphosphonate clodronate is
775 effective in increasing BMD at the lumbar spine in subclinical Cushing's.¹⁵⁴ No guidelines are
776 available for use of possible medical therapies in this particular situation. The use of treatment
777 should also consider that the most common site of fracture in this condition is the spine, BMD can
778 be selectively reduced at the spine and bone density may not fully predict fracture risk associated
779 with glucocorticoid excess. Fracture risk calculators such as FRAX and the Garvan Fracture Risk
780 calculator are based on hip density and might underestimate the risk of vertebral fractures in this
781 condition.

782 It remains unclear whether patients with post-menopausal and age related osteoporosis and no
783 symptoms of hypercortisolemia should be tested routinely for the condition. A pragmatic approach
784 at the current time would be to test those individuals who have a higher likelihood of cortisol excess
785 e.g. people presenting with vertebral fractures, people with BMD values that are highly discordant
786 between spine and hip with spine being low, and people with non-traumatic fractures that occurred
787 in the context of relatively normal bone density. The most appropriate test to identify people with
788 excess cortisol secretion that is likely to impact on bone would appear to be the 1mg DST with a cut-
789 off of 2 micrograms per decilitre (55 nmol/L) (although these values should be adjusted based on the
790 performance of the local cortisol assay).^{7,150,151} Clearly the distinction between autonomous cortisol
791 secretion and overt Cushing's syndrome might be difficult in these situations where there is clear cut
792 bone disease in association with abnormal cortisol secretion. In these situations additional
793 investigations are required to determine the basis for the abnormal cortisol levels.

794

795 III.I.III Bone impact of physiological variation in the HPA axis.

796 It is possible that individual variations in the circulating level of endogenous glucocorticoids might
797 also have an impact on bone even in the absence of any disease or condition affecting the HPA axis.
798 By examining healthy post-menopausal women before and after treatment with the adrenal
799 corticosteroid synthesis inhibitor metyrapone it has been established that the circadian variation in
800 serum osteocalcin is influenced by adrenal cortisol secretion.¹⁵⁵ In the same study, other bone
801 formation or resorption markers did not appear to be influenced by adrenal function suggesting a
802 specific sensitivity of osteocalcin to glucocorticoids independent of its role as a marker of bone
803 formation. Whether variations in adrenal cortisol secretion impacts on bone health has been
804 primarily examined in studies looking at serum or salivary cortisol levels and differences in bone
805 health (mostly assessed as BMD by DXA) during ageing. These studies have generally found weak
806 associations between levels of circulating glucocorticoids at various time of day and either current

bone density or change in bone density over time. The results also appear to differ depending on whether women or men are studied. In the a study of 228 elderly community dwelling people salivary cortisol levels at 2300 were negatively associated with lumbar spine BMD in women whereas in men 0700 salivary cortisol levels negatively correlated with spine BMD.¹⁵⁶ In 34 healthy elderly men that had frequent serum cortisol measurements over a 24 hour period the integrated serum cortisol level over the 24 hour period was negatively associated with lumbar spine BMD. Additionally, trough cortisol predicted the rate of bone loss at the spine and femoral neck over the subsequent 4 years.¹⁵⁷ In a study of over 500 men and women from the Longitudinal Ageing Study Amsterdam serum fasting cortisol was associated with lower BMD at the femoral neck after adjustment for age and BMI.¹⁵⁸ A study of 135 elderly women and 171 men examined the relationship between serum cortisol, serum cortisone, bone markers and BMD.¹⁵⁹ It was found that serum cortisol had no relationship with any bone measurements but serum cortisone was negatively associated with serum osteocalcin levels and spine BMD. These relationships were independent of the levels of cortisol. This study suggests that the relationship between adrenal corticosteroid production and bone health may, at least in part, be mediated via cortisone. The only other study that explored the role of cortisone in bone health performed a comprehensive analysis of adrenal corticosteroid output and metabolism in young males in relation to bone development at the proximal radius. In this study the level of urinary cortisone metabolites was independently and negatively associated with reduced bone density.¹⁶⁰

A significant limitation of these studies is their lack of information relating to fractures. Two studies have however provided information in relation to fracture risk and adrenal corticosteroid production. A sub-study of the MacArthur Study of Successful Ageing measured overnight (between 2000 to 0800 hrs) urinary free cortisol excretion in 684 men and women aged 70-79 at baseline.¹⁶¹ Higher baseline UFC was significantly associated with the incidence of self-reported fractures over the next 4 years. These relationships appeared to be relatively strong e.g. in the highest quartile of UFC the adjusted odds of a fracture was over 5. A more recent cross-sectional study examined the

relationship between salivary cortisol measurements taken at various times of the day and the TBS and presence of vertebral fractures.¹⁶² The study involved over 600 women and vertebral fractures were defined on the basis of Genant grade 2 or greater fractures on spine radiographs. This criteria (a loss of height of greater than 25%) is more stringent than that typically used in the studies examining spine fractures in people with subclinical Cushing's described above and would be expected to increase the clinical significance of these fractures. It was found that salivary cortisol levels at 2000 hrs were associated with the presence of vertebral fractures and that this relationship was independent of age and BMD. A negative linear association between 2000 hrs salivary cortisol and TBS values was also observed. Morning salivary cortisol levels were not found to be associated with fracture prevalence. In multivariable models both evening salivary cortisol levels and TBS scores independently predicted the presence of a spine fracture. Although methodologically very different these two studies strongly support the idea that high exposure to endogenous cortisol levels in the evening and overnight, even within the normal range, is associated with an increased risk of fracture.

III.I.IV Bone impact of variation in glucocorticoid receptor expression.

A further possible way in which endogenous glucocorticoid action within bone could be amplified is an alteration in the sensitivity or number of the glucocorticoid (or mineralocorticoid) receptors within the cell or an alteration in post-receptor signalling. Several studies have examined the influence of GR gene (NC3R1) polymorphisms on the sensitivity of bone to glucocorticoids. These have generally been small and either negative or reported weak and inconsistent associations. Huizenga et al examined the influence of the N363S polymorphism of the GR gene on various aspects of glucocorticoid sensitivity and bone composition.¹⁶³ Heterozygous carriers of this polymorphism had greater suppression of serum cortisol levels during a 0.25mg overnight DST implying greater sensitivity at the level of the GR. In terms of bone density there was a non-

858 significant difference of approximately 0.5 of a Z-score at the spine ($p=0.08$) but no suggestion of a
859 difference at the hip. This study was additionally limited by the low number of people with the
860 N363S polymorphism at just 10 compared to over 100 controls without. The gene for the GR has not
861 been linked to osteoporosis or fracture risk in genome wide association studies suggesting that
862 variation in the GR is unlikely to be a major factor in the development of these conditions. A possible
863 reason for this lack of association is that relatively modest changes in GR sensitivity are unlikely to
864 have consequences as long as normal HPA negative feedback is intact. Any difference in sensitivity
865 would be expected to be compensated for by small changes in circulating levels of cortisol.

866 GR gene variants that influence glucocorticoid sensitivity could influence the degree of bone damage
867 that occurs in people with excessive adrenal cortisol production due to disease states or exogenous
868 glucocorticoid usage. In these situations the HPA negative feedback would be unable to adjust for
869 difference in glucocorticoid sensitivity. Studies in these situations have suggested a possible impact
870 of GR variants. Szappanos et al. examined several GR gene variants (N363S, Bcll, ER22/23EK and
871 A3669G) in 60 people with endogenous Cushing's syndrome and 129 healthy controls.¹⁶⁴ They found
872 that individuals with Cushing's syndrome that were homozygous for the Bcll polymorphism had
873 reduced BMD at the hip by DXA and an increased level of serum betaCTx (a bone resorption marker).
874 The other polymorphisms did not appear to influence bone. Koetz et al. examined the influence of
875 the Bcll polymorphism in 112 patients with adrenal insufficiency.¹⁶⁵ Patients homozygous for the G
876 variant (which would be expected to increase cellular glucocorticoid sensitivity) were found to have
877 greater serum betaCTx and greater urinary NTx. However there was no difference in BMD at hip or
878 spine. Interestingly these patients were treated with significantly lower doses of replacement
879 glucocorticoids. This lower dose may have offset the increased tissue glucocorticoid sensitivity.

880 It might also be hypothesised that variants in GR sensitivity would predict the effects of therapeutic
881 glucocorticoids on bone. However, these studies are likely to be complicated by any impact that
882 variation in GR sensitivity might have on the activity of the underlying disease being treated. For

883 example, carriers of the N363S or Bcll minor variants (which predict increased glucocorticoid
884 sensitivity) are reported to have a lower risk of developing rheumatoid arthritis.¹⁶⁶ Likewise, patients
885 with rheumatoid arthritis that are carriers of the Bcll or N363S variants have lower levels of baseline
886 disease activity even in the absence of glucocorticoid treatment.¹⁶⁷ It seems likely that any
887 differences in GR sensitivity through genetic polymorphism will alter bone sensitivity in parallel to
888 that of the underlying disease requiring treatment.

889

890 Another mechanism by which glucocorticoid action could be altered at a tissue level is through the
891 active transport of glucocorticoids across cell membranes. Several membrane transporters can
892 remove certain types of glucocorticoids from the cytoplasm. This is best exemplified by the active
893 transport of the synthetic glucocorticoid dexamethasone by cells of the blood brain barrier.¹⁶⁸ More
894 recently the selective transport out of certain tissues of cortisol and corticosterone by ABC
895 transporters has been highlighted.⁹ The clinical relevance of these effects is yet to be fully
896 established and they have not yet been examined in the context of bone cells or GIOP.

897

898 III.II Tissue specific amplification of glucocorticoid action

899 Traditionally glucocorticoid action at a tissue level has been assumed to be closely linked with the
900 levels of glucocorticoids in the circulation. More recently it has become apparent that there are
901 additional potential levels of regulation between the circulation and action at a tissue level. The
902 most extensively examined of these levels is that of tissue 'pre-receptor' glucocorticoid metabolism.
903 Various enzymes capable of glucocorticoid metabolism are present within bone. The enzymes that
904 have previously been examined include the 11 β -HSDs and 5 α -reductases. Although expression of
905 the 5 α -reductase type 1 enzyme has been reported¹⁶⁹ the activity of this enzyme in human bone
906 appears modest and more attention has focussed on the 11 β -HSDs.^{10,170} There are two 11 β -HSD

907 enzymes. 11 β -HSD1 is an intrinsically bidirectional enzyme which interconverts hormonally inactive
908 cortisone (human) and dehydrocorticosterone (DHC) (rodent) with their active counterparts cortisol
909 and corticosterone respectively.¹⁰ Although bidirectional in most situations in vivo the enzyme acts
910 principally as an activating enzyme due to the presence of a cofactor generating enzyme hexose-6-
911 phosphate dehydrogenase.¹⁷¹ This enzyme provides a supply of NADPH within the endoplasmic
912 reticulum where 11 β -HSD1 is located. 11 β -HSD2 by contrast is a powerful glucocorticoid inactivating
913 enzyme converting active cortisol and corticosterone to inactive cortisone and DHC. 11 β -HSD2 is
914 normally expressed in classical mineralocorticoid sensitive tissues such as kidney, colon and
915 pancreas, where it protects the MR from binding by glucocorticoids, whilst 11 β -HSD1 is more widely
916 expressed in tissues such as liver, adipose and skin. In terms of expression within bone, 11 β -HSD
917 activity was first recognised in cultured osteosarcoma cells and primary cultures of osteoblasts.^{172,173}
918 In osteosarcoma cells 11 β -HSD2 mRNA and activity were detected whereas primary cultures of bone
919 demonstrated exclusive expression of 11 β -HSD1¹⁷³. It is now known that 11 β -HSD2 is expressed in a
920 range of malignant tissues and its presence in osteosarcoma cells is thought to reflect their
921 malignant status rather than being a feature of bone cells.^{60,174} Studies in adult mouse and human
922 bone demonstrate expression of 11 β -HSD1 but not 11 β -HSD2.^{59,175} Immunohistochemistry and in
923 situ hybridisation studies demonstrated that the main cell type expressing 11 β -HSD1 in bone were
924 osteoblasts and osteocytes¹⁷⁵. 11 β -HSD1 expression was seen to a lesser degree in osteoclasts.¹⁷⁵ In
925 vitro expression appeared to vary across osteoblast differentiation with levels being low in immature
926 cells, rising and reaching a peak in mature osteoblasts.¹⁷⁶ The functional significance of 11 β -HSD
927 expression in bone cells was examined by transfection and stable expression of these enzymes in
928 osteosarcoma cell lines which do not normally have 11 β -HSD activity.¹⁷⁷ Whereas empty vector cells
929 were unresponsive to cortisone, expression of 11 β -HSD1 rendered cells sensitive to cortisone in
930 terms of reduced proliferation and expression of glucocorticoid responsive bone cell markers.

Comment [M6]: Should have a figure here.

931 The expression and activity of 11 β -HSD1 have been shown to be regulated by age, cell
 932 differentiation status, inflammation and by glucocorticoids themselves. Primary cultures of human
 933 osteoblasts demonstrated greater ability to generate cortisol from cortisone when cells were grown
 934 from older compared to younger donors.¹⁷⁸ This relationship was also observed in mice where mRNA
 935 for 11 β -HSD1 was increased in bones obtained from old compared to young mice.⁷⁷ The
 936 inflammatory cytokines TNF α and IL-1 β are powerful stimulators of 11 β -HSD1 activity in
 937 mesenchymal derived cell populations such as osteoblasts, and have been proposed as potential
 938 mediators of increased 11 β -HSD1 activity in aging.^{179,180} This upregulation appears to be via an NF- κ B
 939 dependent mechanism, although CCAAT/enhancer-binding protein (C/EBP) β has also been shown to
 940 play a role in this inflammatory induction of 11 β -HSD1.¹⁸⁰⁻¹⁸² Glucocorticoids themselves also cause a
 941 modest increase in 11 β -HSD1 activity and expression in osteoblasts and they can synergise with pro-
 942 inflammatory cytokines to cause a more dramatic increase in 11 β -HSD1 expression.^{178,183}

943 Clinical studies also indicate the presence of 11 β -HSD1 within bone. In a cohort of elderly subjects
 944 the level of cortisone in the circulation was a significant negative predictor of the blood level of
 945 osteocalcin whereas cortisol was not.¹⁵⁹ This suggested that 11 β -HSD1 within osteoblasts is a
 946 regulator of osteocalcin synthesis. A number of relatively small genetic association studies have
 947 suggested that polymorphisms in the 11 β -HSD1 gene (HSD11B1) might contribute to the
 948 development of osteoporosis, regulate the level of serum osteocalcin, or increase the risk of
 949 fracture.¹⁸⁴⁻¹⁸⁷ However, the gene has not been identified as a candidate in large GWAS studies. It is
 950 possible that these polymorphisms might be important in some ethnic groups but not others. It also
 951 needs to be considered that 11 β -HSD1 is also expressed in other tissues and any associations could
 952 be mediated indirectly e.g. through an effect on the regulation of the degree of inflammation, rather
 953 through an effect on bone cells themselves.

954 The functional role of 11 β -HSD1 in bone has been examined in some animal models. In mice with
 955 global deletion of 11 β -HSD1 there is no alteration in bone density or structure.¹⁸⁸ However, on the

background examined the 11 β -HSD1 global knock out mice have an alteration in feedback regulation of the HPA axis leading to a high level of corticosterone in the circulation.¹⁸⁹ It is possible that this high circulating level might offset any tissue level reduction in glucocorticoid levels. The global knockout mouse has not been evaluated in the context of old age or in models of glucocorticoid excess and inflammation associated osteoporosis. Certainly, in the context of glucocorticoid induced muscle wasting, skin thinning and hepatic steatosis, global deletion of 11 β -HSD1 results in almost complete protection raising the possibility that these mice will also be protected from glucocorticoid induced osteoporosis.

The 11 β -HSD enzymes also regulate the activity of the most widely used oral glucocorticoids prednisone and prednisolone.¹⁹⁰ 11 β -HSD1 converts inactive prednisone to active prednisolone with similar enzyme kinetics to that of the conversion of cortisone to cortisol. In healthy males the baseline level of 11 β -HSD1 (measured as the ratio of corticosteroid metabolites on a 24 hour urine collection) predicted the response of bone formation markers to a short course (7 days) of oral prednisolone. High baseline 11 β -HSD1 activity was associated with the greatest falls in serum osteocalcin and PINP levels. This relationship was independent of the circulating levels of prednisone or prednisolone. The conclusions from this study are limited due to the activity being measured in the total body rather than in the bone itself. Additionally, even though the predictive ability of total measures of 11 β -HSD1 activity are predictive of the response of bone to glucocorticoids these relationships may not persist in patients treated with glucocorticoids for inflammatory disease since inflammation itself is associated with a tissue specific increase in 11 β -HSD1 activity.¹⁹¹ In patients with inflammatory bowel disease baseline measures of 11 β -HSD1 activity on a urine sample were not predictive of the change in bone density in response to oral glucocorticoid treatment.¹⁹²

Given that inflammation increases 11 β -HSD1 activity and activation of therapeutic glucocorticoids within bone cells, the potential exists for locally activated steroids to both abrogate inflammatory bone loss whilst directly contributing to glucocorticoid mediated bone loss. Clinical data suggest that

the reality may lie somewhere in-between the two, with therapeutic glucocorticoids partially suppressing disease activity in patients with chronic inflammatory disease and reducing immediate bone loss whilst ultimately contributing to glucocorticoid-induced osteoporosis with prolonged use.^{193,194} These data support the idea that a rapid and marked increase in 11 β -HSD1 in response to inflammation is an important part of the host response to inflammation, with elevated glucocorticoid activation preventing inflammatory bone loss in an acute setting. This situation is complicated in chronic inflammation, where prolonged increases in 11 β -HSD1 may begin to promote bone loss in a similar fashion as seen with long term therapeutic glucocorticoid application.

If correct, the targeted inhibition of 11 β -HSD1 in an inflammatory context may be highly disadvantageous, in a similar manner as reported in the muscles of mice with systemic inflammation on a 11 β -HSD1 KO background.¹⁹⁵ Here, the reduction in local steroid activation within muscle greatly increases systemic inflammation and local muscle inflammatory cytokine production, increasing inflammatory catabolic and anti-anabolic muscle wasting. In a similar manner, systemic inhibition of 11 β -HSD1 may exacerbate inflammatory bone loss. Instead, alternative approaches, for example, targeted inhibition of pro-inflammatory NF- κ B (or any other tissue specific regulator of 11 β -HSD1 activity) or bone selective inhibition of 11 β -HSD1 may be a more effective approach.

IV Therapeutic glucocorticoid excess and bone

This section reviews data relating to the epidemiology and treatment of iatrogenic GIOP. Importantly, as discussed throughout this article, the term GIOP in this context could be misleading. This is because therapeutic glucocorticoids might indeed lead to bone loss through their direct actions but are also likely to have complex interactions with the underlying disease being treated.^{5,6} Glucocorticoids in some situations might magnify the amount of damage being done to bone through worsening of imbalances between bone formation and resorption but in conditions

1005 characterised by systemic inflammation, glucocorticoids, particularly when used at modest doses
1006 might be 'bone sparing' through their anti-inflammatory actions.^{196,197} In the later situation bone
1007 disease might be present in the context of prolonged glucocorticoid use but the damage would not
1008 be truly 'glucocorticoid-induced'. It is generally thought that most systemic inflammatory illnesses
1009 cause bone loss primarily through increased bone resorption with a relative suppression or restraint
1010 on bone formation.^{6,198} The effectiveness of different treatment approaches (anti-resorptive agents
1011 targeting osteoclast activity vs. anabolic drugs targeting bone formation) might depend on the
1012 extent to which bone disease is secondary to inflammation or to the glucocorticoids needed to
1013 control the underlying disease.

1014 IV.I Epidemiology of glucocorticoid use and impact on bone

1015 The use of therapeutic glucocorticoids in the community is still high and may even be increasing.
1016 Studies around the turn of the century from the UK reported that up to 1% of the population were
1017 taking oral glucocorticoids on a long term basis.^{199,200} This figure rose to almost 3% in the elderly.
1018 Data from the US based on the NHANES database between 1999 and 2008 estimated that the
1019 prevalence of long term use was 1.2%.²⁰¹ In the Global Longitudinal Study of Osteoporosis in Women
1020 (GLOW) the rate of glucocorticoid usage at baseline study visit in this post-menopausal population
1021 was 4.6%.²⁰² Studies based on UK databases indicate that the rate of long term glucocorticoid use is
1022 gradually increasing.²⁰³ A recent study based on the population of Denmark reported that 3% of the
1023 Danish population filled at least one prescription for a systemically administered therapeutic
1024 glucocorticoid. In the Danish elderly population this figure rose to around 8-10%.²⁰⁴ As such a
1025 significant proportion of the global population is exposed to therapeutic glucocorticoids.
1026 Several studies have attempted to estimate the fracture risk associated with long term
1027 glucocorticoid use. In some groups of patients treated long-term with oral glucocorticoids, the risk of
1028 developing osteoporosis and vertebral fractures was estimated at 50% or more.⁴ These rates will
1029 depend on the specific disease being treated and the age and gender profile of the populations

1030 studied. Population based studies have similarly indicated that glucocorticoid usage is associated
1031 with an increased risk of fracture.³ Importantly, risks of fracture were increased at the hip (relative
1032 risk increase 1.6) and spine (relative risk increase 2.6) as well as an increased risk of non-vertebral
1033 fractures (relative risk 1.3). Even relatively modest doses of glucocorticoids were associated with a
1034 significantly increased fracture risk, with doses as low as 2.5mg/day being linked to spine fractures.
1035 Risk of fractures was also associated with daily dose with a 20% increased risk of fracture seen at 5
1036 mg/day of prednisolone, increasing to 60% at 20 mg/day. The time of onset and offset of fracture
1037 risk was particularly instructive. In the study by van Staa et al. the risk of fracture increased rapidly
1038 within a short time of commencing glucocorticoid therapy.³ The risk of fracture remained elevated
1039 while glucocorticoids were continued but fell rapidly after glucocorticoids were ceased. The
1040 mechanisms for these rapid changes in fracture risk are unclear but changes in bone density alone
1041 are an unlikely explanation. An increased risk of falls due to myopathy associated with glucocorticoid
1042 use might be part of the explanation. The risk of fracture also appeared to increase in glucocorticoid
1043 users even before therapy was initiated, indicating that the indication for treatment is an important
1044 component of the increase in risk of fracture during therapy. As such glucocorticoid treatment is
1045 likely to be a marker of the presence of a disease associated with increased fracture risk as well as an
1046 independent factor itself. Support for this involvement of underlying disease in fracture risk comes
1047 from studies of patients taking inhaled glucocorticoids for respiratory disease, who had a higher
1048 fracture risk than healthy matched controls.²⁰⁵ However, patients taking inhaled bronchodilators but
1049 no inhaled glucocorticoids had a similar increase in fracture risk compared to controls, a finding that
1050 implies that the underlying respiratory condition was the most significant contributor to fracture
1051 risk.

1052 Subsequent studies have attempted to determine whether fracture risk was associated with
1053 cumulative or daily dose. In general both dose and duration influence fracture risk and these two
1054 factors are difficult to separate out in clinical practice.^{206,207} The question was recently addressed in a
1055 population based cohort of over 50,000 patients from Canada which examined the relative

Comment [M7]: Table for epidemiology studies?

1056 importance of recent or remote and short or prolonged use of glucocorticoids on bone density and
1057 fracture incidence.²⁰⁸ In this cohort only recent prolonged glucocorticoid use was associated with
1058 reduced femoral neck T-scores and a BMD-independent increase in the risk of major and hip
1059 fractures. However, most other studies provide reassurance that glucocorticoid use is only harmful
1060 to bone when used for relatively long durations. Occasional intermittent use of high dose
1061 glucocorticoids has been reported to be relatively safe in terms of bone health.²⁰⁹ However, a recent
1062 retrospective cohort study based on private insurance claims from the US demonstrated an
1063 association between short-term glucocorticoid use (less than 30 days) with various types of harm
1064 including a 1.8 fold increased risk of fracture.²¹⁰ Such short-term use of glucocorticoids was common
1065 at 20% of the population over the 3-year period examined, suggesting that intermittent
1066 glucocorticoid use could contribute more to population fracture risk than previously thought.

1067 Another important source of information relating to the epidemiology of GIOP are the placebo arms
1068 of the initial RCTs that examined the impact of various treatments on the development of GIOP.²¹¹⁻
1069 ²¹³ This approach has the advantage of having greater sensitivity for determining the impact of
1070 glucocorticoids on vertebral fracture risk as spine radiographs were typically taken in these trials.
1071 Overall, the placebo arms of these studies indicate that glucocorticoid treatment is associated with a
1072 high risk of fracture, particularly vertebral fracture in post-menopausal women and older men.^{101,214}
1073 However, the risk of fracture in pre-menopausal women and younger men appeared to be very low.
1074 Where fractures did occur the BMD T-score tended to be below a level of -1.5. Whether these
1075 younger patient groups have reduced absolute risks of fracture by virtue just of their age or whether
1076 there are independent age related protective factors such as sex steroid levels remains unclear. A
1077 recent formal meta-regression of data from the placebo arms of these studies has reported annual
1078 incidence rates of vertebral fracture of 5.1% and 3.2% for patients initiating or continuing
1079 glucocorticoid treatment respectively.²¹⁴ The corresponding rates of non-vertebral fractures were
1080 2.5% and 3.0%.

1081 Other studies have attempted to define the effect of glucocorticoid treatment on bone density and
1082 architecture over time. Zhu et al. performed a carefully controlled prospective study of women with
1083 SLE on long term glucocorticoids followed up for over 2 years.²¹⁵ Areal BMD by DXA at multiple sites
1084 and microstructural analysis by high resolution peripheral QCT (HR-pQCT) at the distal radius were
1085 examined at baseline, 12 and 24 months. In premenopausal women the changes in aBMD by DXA
1086 over the two years were very similar between cases and controls. There was however a significant
1087 decrease in cortical area and thickness and an increase in cortical porosity in cases compared to
1088 controls. In post-menopausal women, again there was no significant difference in changes in BMD
1089 between cases and controls but by HR-pQCT there was a significant decrease in volumetric BMD at
1090 the cortex and more substantial decreases in cortical thickness and increases in cortical porosity. The
1091 increases in porosity seen were double in post-menopausal women compared to premenopausal
1092 women and the decrease in cortical thickness 10 fold greater. As such, at least in SLE, cortisol bone
1093 loss is significant during glucocorticoid treatment with the magnitude of changes being substantially
1094 greater in post-menopausal women. This data indicate that prolonged use of glucocorticoids leads to
1095 deterioration in bone, and in particular cortical architecture, even in the absence of any changes in
1096 BMD measured by DXA. A similar DXA independent deterioration in bone quality may also occur at
1097 the spine. Paggiosi et al. compared spine aBMD by DXA and TBS values in 484 women treated with
1098 or without glucocorticoids.²¹⁶ There was no difference in aBMD between groups but the TBS Z-score
1099 was 0.8 lower in the group treated with glucocorticoids. Whether the change in TBS is predictive of
1100 fractures in this situation is not yet clear.

1101 An important consideration in these studies is whether the changes in trabecular or cortical bone
1102 seen in people treated with glucocorticoids is due to the glucocorticoids or to the underlying disease
1103 being treated. Some studies suggest that the underlying disease itself could be contributing more to
1104 the adverse effects on bone than glucocorticoids. For example Olsson et al. examined the effect of
1105 short term, high dose glucocorticoids on bone in patients with multiple sclerosis.²¹⁷ No independent
1106 association was found between glucocorticoid usage and BMD. However, disease activity was

1107 strongly associated with decreases in spine and hip BMD. In a population based cohort study
1108 examining 1 million patients with or without COPD, COPD severity was strongly related to an
1109 increased risk of osteoporosis and fracture.²¹⁸ However, prednisolone use and inhaled corticosteroid
1110 use were associated with a reduced rather than increased risk of osteoporosis. Although this type of
1111 study could be influenced by confounding this is further evidence of the importance of the
1112 underlying disease on fracture risk in glucocorticoid treated patients. Even though reductions in
1113 BMD in patients treated with glucocorticoids may be more strongly related to the underlying disease
1114 in some circumstances than the glucocorticoids themselves, from a practical point of view BMD
1115 measurements are still clinically useful as these patients are likely to be at an increased risk of
1116 fracture and likely to benefit from treatment.

1117

1118 IV.II Risk stratification in the clinical setting

1119 As with guidelines relating to other forms of osteoporosis there has been a shift away from fixed cut
1120 off values of BMD by DXA as the basis for estimation of risk to the use of calculators such as FRAXTM
1121 ²¹⁹and the Garvan Fracture Risk calculator²²⁰ that include multiple aspects of fracture risk. Currently
1122 guidelines for GIOP risk stratification attempt to focus on estimated absolute risk but usually include
1123 additional 'red flags' that would prompt treatment even if the estimated risk is below the
1124 intervention threshold. A question relating to exposure to glucocorticoids is incorporated into
1125 FRAXTM, the most widely used fracture risk calculator, but not other fracture risk calculators. The
1126 glucocorticoid question requires a yes/no answer with the yes answer indicated if the patient is
1127 currently exposed to oral glucocorticoids or has been exposed to oral glucocorticoids for more than
1128 3 months at a dose of prednisolone of 5mg daily or more. As discussed earlier, the risks associated
1129 with glucocorticoids in epidemiological surveys indicate that current treatment with glucocorticoids
1130 is a more powerful risk factor for fracture than remote use and the relationship is also, to some
1131 extent, dose dependent. To address this additional guidance is now available on how to adjust the

1132 FRAX output manually based on glucocorticoid dose and recency of exposure.²¹⁹ A more
1133 fundamental limitation of FRAX and other calculators in the context of GIOP is the focus on hip and
1134 non-vertebral fractures with little emphasis on spine fractures. This extends to the requirement to
1135 enter femoral neck BMD with no possibility of entering spine BMD scores. As such in younger
1136 patients with relatively preserved BMD it is unlikely that high fracture risk values will be generated
1137 even if the risk of spine fractures was clinically significant. To address this, many guidelines also
1138 suggest treatment to be considered if the patient has a low traumatic fracture, or has a low BMD e.g.
1139 less than -1.5 T score by DXA, particularly at the spine. Although not evaluated in the context of
1140 clinical studies it would seem reasonable clinical practice to have a lower threshold for obtaining
1141 spine radiographs or VFA by DXA (if available) in patients at risk of GIOP.

1142

1143 IV.III Treatment strategies

1144 There are a range of guidelines and recommendations published for the pharmacological treatment
1145 of GIOP.²²¹⁻²²³ Intervention thresholds and rules for treatment use and patient reimbursement vary
1146 considerably between countries. These guidelines will not be reviewed extensively here but rather
1147 the evidence related to the effectiveness of various treatments specifically on fracture risk are
1148 described below.

1149 A number of RCTs have been performed in the context of GIOP. These are generally much smaller in
1150 size than the RCTs that demonstrated the effectiveness of these medications in post-menopausal
1151 osteoporosis and generally were not powered for fracture. The main RCTs that compared
1152 treatments to placebo include those that evaluated etidronate,²¹² alendronate²¹³ and risedronate,²¹¹
1153 whereas zoledronic acid²²⁴ and teriparatide²²⁵ evaluated medications in non-inferiority studies.
1154 These trials consistently demonstrated improvements in BMD by DXA relative to placebo in patients
1155 treated shortly after initiation of glucocorticoids and patients treated with glucocorticoids prior to

1156 initiation of therapy. Although not powered for fracture risk reduction, post-hoc analyses have
1157 indicated the likely impact of these treatments on fracture risk, particularly at the spine.^{226,227} Other
1158 studies have examined the effect of vitamin D or its metabolites but these have generally been
1159 smaller and found less substantial changes in BMD.²²⁸⁻²³⁰ Due to heterogeneity of inclusion criteria,
1160 baseline fracture risk and methods of ascertainment of BMD and fracture incidence it is difficult to
1161 compare between treatments. However, the effectiveness of alendronate and teriparatide has
1162 been compared in the context of non-inferiority. Saag et al. compared teriparatide with alendronate
1163 treatment over 18 months in a randomised double blind study of 428 women and men receiving
1164 therapeutic glucocorticoids.²²⁵ Spine BMD by DXA increased substantially more in the teriparatide
1165 group than in the alendronate group. Most importantly, in a pre-specified secondary analysis, the
1166 risk of developing new morphometric vertebral fractures were considerably lower in patients taking
1167 teriparatide (0.6%) than alendronate (6.1%). These results are dramatic especially taking into account
1168 that alendronate substantially reduces the risk of new morphometric vertebral fractures when
1169 compared to placebo.²²⁶ The superiority of teriparatide compared to bisphosphonates for increasing
1170 bone strength at the spine is also supported by an open label randomised trial of teriparatide versus
1171 and risedronate in men treated with glucocorticoids.²³¹ This smaller RCT (92 men in total) focussed
1172 primarily on spine BMD and structural parameters using DXA, QCT of L1-3, and high resolution QCT
1173 or the T12 vertebra. Both treatments led to improvements in various parameters of bone density
1174 and strength but these were in general much greater with teriparatide. Estimates of vertebral
1175 strength using finite element analysis (FEA) demonstrated clear superiority of teriparatide over
1176 risedronate. Even though small, there was also a trend towards fewer spine fractures in men treated
1177 with teriparatide compared to those treated with risedronate. The effects of alendronate versus
1178 teriparatide on spine TBS have also been compared in a secondary analysis of a RCT of these
1179 treatments in GIOP.²³² It was found that alendronate had no impact on TBS despite increasing aBMD
1180 whereas teriparatide caused a significant increase in TBS in addition to positive impact on aBMD.
1181 These data collectively indicate that the bisphosphonates and teriparatide are effective in reducing

1182 fracture risk at the spine in GIOP but teriparatide appears superior over 18 months. An important
 1183 caveat regarding teriparatide is that it is generally only licenced for a duration of 18 months
 1184 (depending on country) and subsequent anti-resorptive medications are typically needed after this
 1185 treatment ends.

1186 The small size of these RCTs and relative rarity of non-vertebral and hip fractures compared to
 1187 morphometric spine fractures means that the effect of these drugs on non-vertebral and hip fracture
 1188 risk is not possible to determine. Three recent publications have used retrospective database
 1189 analysis to try to determine the real world impact of these medications on overall fracture risk and
 1190 hip fracture risk specifically. Overman et al. examined the effect of anti-osteoporotic medications
 1191 (AOMs, including bisphosphonates, teriparatide and denosumab) collectively on the risk of clinical
 1192 fractures in women aged 50 plus taking oral glucocorticoids included in the MarketScan databases.
 1193 The analysis included 7885 women with 12.1% of them treated with AOMs. It was found that AOM
 1194 use was associated with significantly reduced hazard ratios (HRs) for fracture of 0.52 at 1 year and
 1195 0.68 at 3 years.²³³ Axelsson et al. examined the association between alendronate use and hip
 1196 fracture risk in women and men using glucocorticoids in a national Swedish database including
 1197 433,195 patients 65 years and older.²³⁴ The use of alendronate was associated with a significantly
 1198 reduced risk of hip fracture (HR 0.35). A third study by Bergman et al. also used Swedish national
 1199 databases which included over 3 million people to compare the impact of alendronate on risk of
 1200 fracture in patients treated with glucocorticoids.²³⁵ They found that alendronate use was associated
 1201 with a 16% reduction in non-vertebral fracture and a 34% reduction in hip fractures compared to
 1202 non-users. These studies clearly have intrinsic limitations due to their non-randomised nature and
 1203 could be influenced by confounding by indication. However, sensitivity analyses within these studies
 1204 consistently supported a strong and clinically important reduction in non-vertebral and hip fracture
 1205 risk in patients taking glucocorticoids who are treated with anti-osteoporosis medications.
 1206 Importantly, these studies also failed to detect any evidence of harm relating to gastrointestinal
 1207 adverse reactions with these medications. These reductions in non-vertebral fracture risk imply that

1208 treatments for GIOP might also improve the strength of cortical bone. A recent study directly
1209 examined in vivo changes in bone tissue properties in glucocorticoid treated patients commencing
1210 various osteoporosis treatments.²³⁶ Reference point indentation (a form of microindentation) was
1211 performed on the tibia using a hand held device under local anaesthetic. This technique measures
1212 the resistance of cortical bone to indentation and thus provides a measure of tissue properties. Over
1213 periods of 7 and 20 weeks treatment with calcium and vitamin had no impact on material properties
1214 (the Bone Material Strength Index; BMSi). However, treatment with risedronate over 20 weeks and
1215 treatment with denosumab or teriparatide over 7 and 20 weeks resulted in a significant increase in
1216 BMSi. There was however no change in BMD by DXA in any group. Although the clinical utility of
1217 microindentation techniques remains uncertain these preliminary studies strongly support a rapid
1218 and beneficial effect of osteoporosis medications on cortical bone properties in patients at risk of
1219 GIOP.

1220 A concern with anti-resorptive drugs is that they predispose to the development of atypical femoral
1221 fractures (AFFs). Although the pathogenesis of AFFs is still unclear decreased bone turnover is
1222 implicated. Since both anti-resorptive medications and glucocorticoids decrease bone turnover
1223 prolonged use of both might theoretically increase the risk of AFFs. Although early reports²³⁷
1224 suggested a possible link between glucocorticoids and AFFs in patients taking bisphosphonates more
1225 recent studies do not support such an association.²³⁸

1226 A 2 year double blind placebo controlled non-inferiority RCT examining the effectiveness of
1227 denosumab compared to risedronate in over 700 women and men with GIOP has been completed
1228 but not published. Data from the 1st year of treatment has been reported in abstract form and the
1229 drug appears to have a positive effect on bone mineral density with increases at spine and hip in
1230 excess of those seen with risedronate. Small observational studies of the use of denosumab in GIOP
1231 suggest that the drug is effective in maintaining or increasing BMD.²³⁹⁻²⁴¹ Formal licencing of
1232 denosumab for this indication is expected in the future.

1233

1234 **V Skeletal impact of glucocorticoid replacement**

1235 This section will review the data relating to the skeletal impact of glucocorticoid replacement for
1236 primary or secondary adrenal insufficiency. The amount of literature in this area is modest but there
1237 have been some recent contributions and the subject is clearly of relevance to an endocrine
1238 audience. When interpreting the literature relating to the impact of glucocorticoid replacement on
1239 bone it is important to consider that patients are treated for long periods of time with some patients
1240 treated for several decades, that treatment regimens have varied over time with a trend to using
1241 lower doses of glucocorticoids in more recent years, and that glucocorticoid replacement may be
1242 only one aspect of their underlying condition that might impact on bone. For example, patients
1243 treated for Addison's disease will additionally have adrenal androgen deficiency and a greater
1244 chance of previous thyrotoxicosis; patients with hypopituitarism will commonly have coexisting
1245 growth hormone deficiency and patients with CAH will commonly have differences in height and
1246 bone structure that make comparisons to controls difficult. Much of the literature focusses on BMD
1247 by DXA but there is some fracture risk data.

1248 The epidemiology of hip fractures in patients with Addison's disease has been examined in a
1249 population based analysis from Sweden.²⁴² Using hospital database, information relating to the
1250 diagnosis of autoimmune adrenal insufficiency and hip fracture of 3,219 patients were identified and
1251 compared to over 31,000 age and sex matched controls from the background population. The risk of
1252 hip fracture was found to be substantially increased with a hazard ratio of 1.8 (CI 1.6-2.1). The
1253 relative risk increase was independent of age or sex. The risk of any fracture was also increased to a
1254 similar degree. The relationship between risk of hip fracture and the time of diagnosis of Addison's
1255 disease was also explored. The relative risk of fracture was most increased in the first year after the
1256 diagnosis but was elevated at all time points. Interestingly, the risk of fracture was also increased by
1257 almost 3-fold in the year prior to the diagnosis, indicating that the cause of the increased fracture

1258 incidence, at least at this time point is not glucocorticoid replacement. It is possible that
1259 glucocorticoid deficiency has a major negative effect on bone in keeping with some of the
1260 observations of an anabolic effect of physiological levels of glucocorticoid action within bone.^{50,63,66}
1261 However, there are additionally many other reasons for this increased fracture risk not least an
1262 increased risk of falls arising from the often severe myopathy seen in untreated adrenal failure.²⁴³
1263 Nevertheless the data support the notion that fracture risk might be increased in patients that are
1264 undertreated as well as those that are over-treated with replacement glucocorticoids.

1265 Other studies have focussed on bone density in Addison's disease. These were mostly cross-sectional
1266 and are difficult to interpret since some of the patients included had been exposed to higher doses
1267 of glucocorticoids than typically used now for prolonged periods of time. In general these studies
1268 reported that adrenal insufficiency was associated with a reduction in BMD at the spine and the hip
1269 and that this reduction was greater with more prolonged use. These studies are summarised in a
1270 recent review by Lee and Greenfield.²⁴⁴ One relatively recent cross-sectional study of patients with
1271 Addison's disease or CAH treated with lower glucocorticoid replacement doses failed to find a
1272 reduction in BMD as assessed by DXA suggesting that replacement regimens adopted more recently
1273 have less negative impact on bone.²⁴⁵ However, another study of 87 patients with Addison's disease
1274 and 81 age and sex matched controls found a higher than expected prevalence of spine fractures
1275 (using DXA based vertebral fracture assessment (VFA)) in patients with adrenal insufficiency despite
1276 there being no difference in BMD.²⁴⁶ Using the Genant criteria 31% of Addison's patients had at least
1277 one vertebral abnormality compared to 12.8% of controls. Suggesting that these fractures might be
1278 related to treatment the risk of fracture appeared greater in those with a longer duration of disease.
1279 Interestingly mineralocorticoid replacement was associated with the presence of a higher BMD.

1280 A recent prospective study examined the impact of targeted reduction in glucocorticoid replacement
1281 dose in patients with Addison's disease and CAH.²⁴⁷ In patients where a reduction in glucocorticoid
1282 replacement appeared justified there was a significant increase in spine and hip Z scores over a 2

1283 year follow up period. This suggests that bone health can be improved by careful attention to
1284 glucocorticoid replacement doses but the actual impact of these changes on risk of fractures has not
1285 been examined.

1286 Several studies have examined the bone health of individuals with CAH and the likely impact of
1287 glucocorticoid replacement.²⁴⁸⁻²⁵² These studies are complicated by the differences in height and
1288 bone size, which tend to overstate reductions in BMD. Additionally, excessive androgen exposure
1289 resulting from inadequate glucocorticoid dosing might have an anabolic effect on bone. However
1290 despite this it appears that bone density is reduced at all skeletal sites in CAH using both DXA and
1291 spinal QCT. The reduction has been correlated with cumulative glucocorticoid exposure in some
1292 studies²⁴⁹ but not others.²⁴⁸ Two small studies by the same research group found that adult women
1293 with CAH had an increased risk of fracture²⁵¹ but men with CAH did not.²⁵²

1294

1295 **VI Future directions**

1296 Currently it is unclear how to separate the anabolic versus catabolic actions of glucocorticoids on
1297 bone. Endogenous glucocorticoids have powerful effects on bone health which appear to some
1298 extent to be regional and surface dependent. In scientific studies there is no consistency in the
1299 regions and surfaces examined which makes comparisons between studies difficult.

1300 There are also limitations with current animal models as it is unclear to what extent they adequately
1301 model the human situation. Few examine the sensitivity of bone in the context of an underlying
1302 illness which is being treated with glucocorticoids. Animal studies examining interventions to protect
1303 against GIOP need to be assessed in inflammatory disease models to determine whether these
1304 interventions have independent actions on the underlying illness being treated in the first place.

1305 It remains unclear if there is a single target for glucocorticoid action within bone that can be
1306 targeted therapeutically. Although there have been attractive targets such as the IGF1 system,
1307 osteoblast apoptosis, IL-11, autophagy, OPG/RANKL and wnts/DKK1/sclerostin no single system
1308 appears to account for all of the actions of glucocorticoids on bone. As discussed above, even if a
1309 single target was identified it would need to be clear that this target was not involved in the
1310 beneficial effects of glucocorticoids on the immune system. Currently the most likely candidates in
1311 this respect are approaches that target sclerostin and/or DKK1 (the other major antagonist of wnt
1312 signalling). The feasibility of such an approach has been demonstrated in principle outside the
1313 context of glucocorticoid therapy in rodents and non-human primates.²⁵³

1314 It should also be remembered that glucocorticoid excess is associated with many adverse effects
1315 outside of bone and these are currently not addressed well. An ideal treatment approach would
1316 target multiple components of risk. Being able to do this without blocking the anti-inflammatory
1317 actions of glucocorticoids has proven difficult. One approach to achieve this goal that has been an
1318 active topic of research for several decades is the development of selective glucocorticoid receptor

1319 modulators. There are various theoretical underpinnings of these molecules largely based on the
1320 concept of dissociating effects of glucocorticoids which are mediated by transactivation and
1321 transrepression.^{254,255} These concepts now appear overly simplistic and furthermore the actions of
1322 glucocorticoids on bone appear to be through transrepression rather than transactivation.⁵⁰ There
1323 have been some interesting compounds examined, in particular 'compound A', which exhibits some
1324 useful features although it's relatively narrow therapeutic range is likely to limit human use.²⁵⁶ Also,
1325 these agents are complicated by the likelihood that they could interfere with HPA axis regulation
1326 leading to low levels of endogenous cortisol. This could create a mixture of excessive glucocorticoid
1327 action in some tissues but an absence of glucocorticoid action in others. Moreover, these
1328 compounds would not exhibit the same properties of selective activation by tissue specific enzymes
1329 and would be unlikely to have effects on the MR (which could play a role in some inflammatory
1330 situations or even modulate the impact of more conventional glucocorticoids on bone). Given these
1331 complexities selective glucocorticoid receptor modulators are unlikely to be developed but if they
1332 are they would probably need to be evaluated in specific inflammatory conditions rather than for
1333 inflammatory disease in general.

1334 There also remains uncertainty regarding what is actually being treated in patients with GIOP. In
1335 some contexts glucocorticoids are likely to be detrimental but in other situations altered bone
1336 remodelling due to the underlying inflammatory disease might be more important. Glucocorticoids
1337 might therefore have an important role in controlling inflammation related bone loss and thus be
1338 bone 'sparing' rather than negative to bone. If this distinction can be made it might be possible to
1339 independently target glucocorticoid induced and inflammation induced bone disease separately or
1340 synergistically.

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